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AN EXPERIMENTAL STUDY OF THE THALAMUS IN THE PHALANGER, *TRICHOSURUS VULPECULA*

By F. GOLDBY, *Department of Anatomy and Histology, University of Adelaide*

In a previous communication the disposition and character of the nuclei and principal fibre tracts in the thalamus of the phalanger were described (Goldby, 1941); it was shown that in this particular diprotodont marsupial the thalamus is essentially like that of primitive mammals in general. A number of progressive features were observed, particularly in the visual system, in the dorsomedial nucleus, and in the ventral nuclei. Subsequent experimental investigations (Packer, 1941) confirmed and extended the conclusions regarding the visual system; in the present work the experimental method had been applied to the remaining parts of the thalamus. Its scope is limited to an examination of the thalamocortical connexions as shown by retrograde degeneration in the thalamus following destructive lesions of the cortex.

MATERIAL AND METHODS

In all, twenty-two adult animals were used, and from nineteen of these satisfactory series of sections were prepared. Sixteen specimens have been selected and will be reported; the remaining three gave no additional information and were useful only in confirming information obtained from the others.

The operations were performed under ether anaesthesia. Small cortical lesions were made with a cataract knife or a meibomian cyst scoop through an incision in the dura just large enough to admit the instrument; most of the larger lesions were made with the diathermy. In nearly all cases damage was inflicted on subcortical fibres as well as on the cellular layers of the cortex, and this has been taken into consideration in assessing the significance of the lesions. The lesions are illustrated both in surface view and in a standard series of twelve transverse sections spaced equidistantly through the hemisphere and numbered anteroposteriorly (Figs. 1–5).

The animals were allowed to survive for periods varying from 6 to 14 weeks, and the brain was removed after injection with 10 % formalin through the aorta. Complete series of transverse sections were cut through the region of the lesion and through the whole thalamus in each case; each alternate section was stained with toluidine blue and mounted; of the remaining sections some were stained by Weil's modification of the Weigert method and the rest stored.

The retrograde degeneration in the thalamus involved a more or less complete loss of nerve cells, gliosis, and, when these changes were at all extensive, marked shrinkage in the nucleus or the affected part of the thalamus (see Pl. 1, fig. 7). No changes were considered significant unless they were observed consistently through several sections, all of which showed an undoubted difference from the normal side. It is often possible in normal brains to find a few cells or small groups of cells which appear shrunken and abnormal, and this is particularly the case in the anterodorsal nucleus. It is probable that this is a fixation artefact rather than a normal cytological feature of the nucleus.

TERMINOLOGY

For the most part the same terminology is used as in the preceding paper on the normal histology of the thalamus (Goldby, 1941). No evidence has been found to justify changing the terminology used in the lateral complex of nuclei; the term 'pars anterior of Bodian' will be used for the small and variable collection of cells which appears to correspond with the nucleus so named by Bodian (1939) in the opossum. The nucleus ventralis posterior was subdivided into dorsomedial and ventrolateral parts separated by a condensation of cells which was compared with the pars arcuata described in some placental mammals. A re-examination of normal material and consideration of the experimental evidence suggest that what was called the dorsomedial part should not be included in the ventral complex; this leaves only two subdivisions in the posterior part of the ventral nucleus ventralis posterior, viz. ventrolateral and pars arcuata. These will be referred to in this paper as the 'pars externa' and the 'pars arcuata' of the nucleus ventralis posterior. It is true that the use of these terms implies homologies which are not established in detail, though as will be seen later, the correspondence with parts of the ventral complex similarly named in other animals is fairly close. Provided their use is not interpreted too strictly, but is judged in the light of the characteristics of these nuclei as reported here and in the previous paper (Goldby, 1941), less confusion should result than from the introduction of entirely new terms applicable only to the phalanger. These remarks apply also to the terminology of other subdivisions of the principal nuclei.

EXPERIMENTAL RESULTS

In order to facilitate comparison the different specimens will as far as possible be described in groups in each of which the cortical lesions have certain features in common. A summary of the principal results obtained in all the specimens is given in the table on p. 209.

The first group contains four specimens, P. 21, P. 16, P. 4 and P. 3 (Fig. 1). In all these the lesion is large, and amounts in P. 21 practically to a hemidecortication so far as the neocortex is concerned.

Experiment P. 21

Survival period: 85 days. A full exposure of the cortex was made from the dorsal aspect and the cortex exposed was destroyed as widely as possible with the diathermy.

The lesion (Fig. 1) has destroyed the whole frontal pole of the hemisphere and opened the lateral ventricle throughout most of its extent, thus cutting off the fibres to or from the medial wall of the hemisphere. A considerable area of cortex above the posterior third of the rhinal fissure remains intact, together with its projection fibres which join the posterior and inferior parts of the internal capsule. This area includes the temporal and a little of the occipital lobes. A few fibres from the medial surface of the occipital lobe have probably escaped as they sweep round on to the lateral surface below the lesion.

There is some damage to the corpus striatum in its anterior two-thirds, and a small part of the lentiform nucleus is destroyed anteriorly, but most of the striatal damage is secondary to vascular disturbance.

Thalamic degeneration

Anterior nuclei (Pl. 1, fig. 7). In the nucleus anterodorsalis about two-thirds of the cells have disappeared, and the loss of cells is practically complete in the posterior half of the nucleus. The nucleus anteroventralis shows almost complete loss of nerve

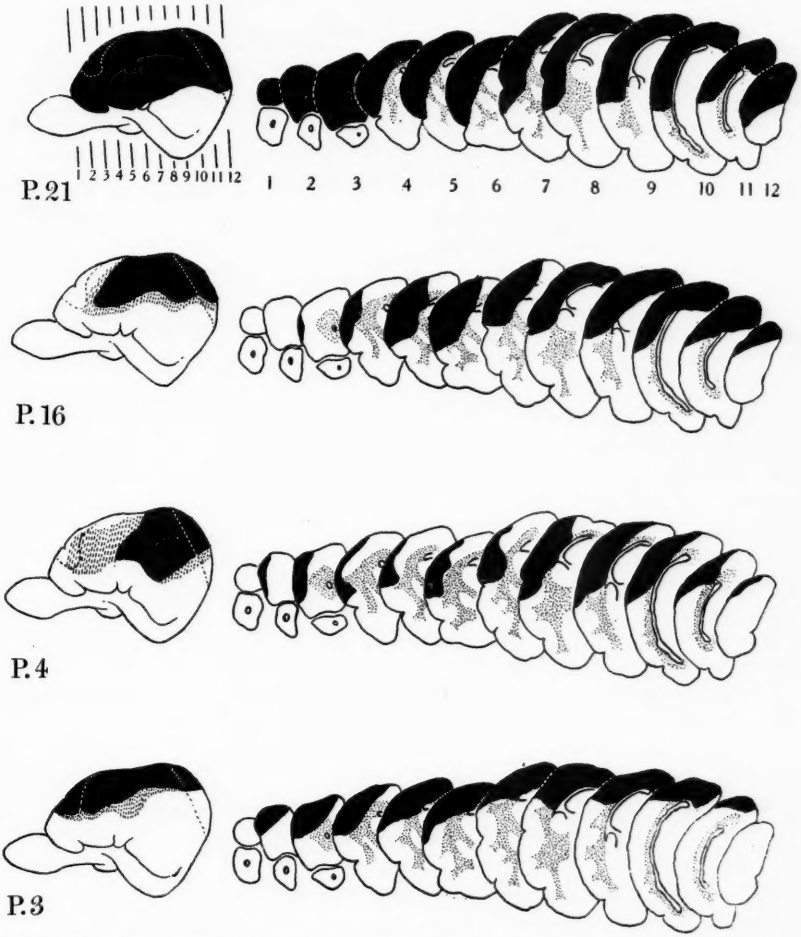

Fig. 1. The lesions in specimens P. 21, P. 16, P. 4 and P. 3. In this and in Figs. 2–5, the superficial extent of the lesion is shown on an outline drawing of the cerebral hemisphere. Solid black shading indicates the area over which the cortex is completely destroyed; shading with interrupted lines indicates superficial damage in which the deeper layers of the cortex are not involved. The sections numbered 1–12 are spaced equidistantly; the plane of section is shown in P. 21, and applies without significant modification to the other specimens in this and other figures. The outline of the motor and visual areas has been marked on the cortex.

cells throughout. The greater part of the nucleus anteromedialis shows changes similar to those in the nucleus anteroventralis, though gliosis is less marked. Dorsomedially there is no degeneration and the normal cells in this situation appear to belong to

the nucleus commissuralis inter-anterodorsalis, which is therefore larger than it was thought to be from a study of normal material. There is also a group of cells, posteromedial to the nucleus anteromedialis, which was included in that nucleus in the previous paper (Goldby, 1941). These also show no signs of degeneration (Pl. 1, fig. 8, *VM*). They appear to correspond with the nucleus ventralis, pars medialis, of the opossum (Bodian, 1939), and this terminology is adopted here.

Dorsomedial nucleus (Pl. 1, fig. 8; Pl. 2, fig. 9). There is almost complete loss of nerve cells and much glial proliferation in the dorsolateral part of this nucleus throughout its length anteroposteriorly.

Ventral nuclei (Pl. 1, fig. 8; Pl. 2, figs. 9, 10). Degeneration is present throughout the nucleus ventralis anterior (Pl. 1, fig. 8). The nucleus ventralis intermedius (Pl. 2, fig. 9) shows rather more intense gliosis, but normal nerve cells are present in two situations: (i) dorsally, beneath the nucleus paracentralis, and (ii) medial to the mamillothalamic fasciculus. The dorsally placed cells may belong to the nucleus paracentralis or may form a nucleus submedius. The medially placed cells could be described as a commissural nucleus since they extend across the mid-line, but actual commissural fibres are very scanty. They should probably be classed with the nuclei of the mid-line.

In the nucleus ventralis posterior, degeneration is complete throughout the pars externa and the pars arcuata (Pl. 2, figs. 9, 10), with particularly intense gliosis in the latter. It does not affect cells dorsal and medial to the pars arcuata, but it has been decided not to include these in the ventral complex (see p. 196).

Geniculate bodies (Pl. 2, fig. 10). Degeneration is practically complete throughout the lateral geniculate body (dorsal nucleus) but a few normal cells are present in its extreme anterior end. The medial geniculate body seems to contain rather fewer cells than on the normal side, but there is no obvious gliosis.

Lateral nuclei (Pl. 1, fig. 8; Pl. 2, figs. 9, 10). Degeneration is marked throughout this complex of nuclei except in the medial large-celled part of the nucleus lateralis posterior, which is unaffected (Pl. 2, fig. 10). For this reason it is impossible to define the boundaries between the subdivisions with any precision.

In the pars anterior (Bodian) (Pl. 2, fig. 9) the cell content is slightly reduced as compared with the normal side, and the remaining cells are shrunken and stain deeply. The nucleus lateralis A contains only a few normal cells, and these are chiefly situated close to its dorsal surface and adjacent to the pars anterior (Bodian) (Pl. 2, fig. 9); apart from this, small pale neurones can be seen, unlike those of the normal nucleus and apparently less numerous. In the nucleus lateralis B degeneration is very similar, but gliosis is more in evidence (Pl. 2, fig. 9). In the nucleus lateralis posterior (Pl. 2, fig. 10) there are two clearly marked subdivisions: a lateral small-celled part, adjacent to the lateral geniculate body, and a medial large-celled part adjacent to the pretectal nucleus. In the lateral small-celled part degeneration is similar in every way to that in nucleus lateralis B. The medial large-celled part is completely normal.

Mid-line, intralaminar nuclei, etc. (Pls. 1, 2, figs. 7–10). No evidence of degeneration could be found in any of these nuclei. The paracentral and lateral central nuclei are more clearly seen than in a normal specimen on account of the degeneration in the adjacent principal nuclei. Actually they appear considerably more bulky than normal material would suggest (e.g. in Pl. 1, fig. 8), extending into the marginal zones of the principal nuclei. It is probable that the same should be said of the nucleus commissuralis inter-anterodorsalis in its relation to the anteromedial nucleus. No defined

centre median nucleus was seen in the degenerate any more than in the normal thalamus. At the same time it may be pointed out that normal cells persist in considerable numbers beneath the paracentral nucleus and extend back on the dorsomedial aspect of the ventral complex to the lateral side of the nucleus parafascicularis (Pl. 2, fig. 10). Anteriorly these cells may represent a nucleus submedius; posteriorly, in their relationship to the pars arcuata of the nucleus ventralis posterior and to the parafascicular nucleus, they resemble a centre median nucleus very closely, and may well form the rudiment from which it is developed in other mammals.

It is clear that in its general features this specimen is very like other mammals in which extensive decortication has been performed. All the principal nuclei show marked degenerative changes, although by no means to a uniform degree. A larger proportion of normal cells persists in the anterodorsal nucleus and the nucleus lateralis A than in any other nuclei. Gliosis, though generally present in degenerated areas, is most intense in the ventral nucleus, particularly in the pars arcuata of the nucleus ventralis posterior. The fact that degeneration is slight or absent in the medial geniculate body is consistent with the lack of damage to the temporal region of the cortex, an interpretation confirmed by a subsequent experiment (P. 48). It is probable that the absence of degeneration from other nuclei or regions of the thalamus cannot be explained similarly.

With very few exceptions no degree of degeneration has been found in any thalamic nucleus greater than in the corresponding nucleus of this specimen (P. 21). In the descriptions of other specimens the term 'complete degeneration' applied without further qualification must be taken as meaning degeneration to the same degree as in P. 21.

Experiment P. 16

Survival period: 79 days. A large area of the dorsolateral cortex was destroyed with the diathermy. The lesion (Fig. 1) has destroyed all layers of the cortex and the subcortical white matter down to the ventricle over most of the area which it covers. The frontal pole of the hemisphere, the motor area, a strip of cortex about 2 mm. wide above the rhinal fissure, the temporal and a small part of the occipital cortex are not directly affected. The fibres to and from the motor area (with the exception probably of its most anterior part) have been divided subcortically, so that the main difference from P. 21 lies in the presence of the frontal pole and of the strip of cortex above the rhinal fissure anteriorly. The projection fibres to both these regions are intact. There is slight direct damage to the anterior part of the caudate nucleus and the putamen.

Thalamic degeneration

Anterior nuclei. There is some loss of cells from the anterior part of the anterodorsal nucleus and the remaining cells are degenerate. The posterior tail of the nucleus is unaffected. The anteroventral and anteromedial nuclei are completely degenerate, except, as in P. 21, for the cells situated dorsomedial and posteromedial to the nucleus anteromedialis.

Dorsomedial nucleus. This nucleus is completely normal.

Ventral nuclei. Degeneration is complete in all parts of the ventral nucleus and does not differ significantly from that in P. 21. The same regions close to the mid-line and beneath the paracentral nucleus are unaffected.

Geniculate bodies. The lateral geniculate body is completely degenerate except for a small anterior part, about one-sixth of the whole. The medial geniculate body again shows slight degenerative changes of doubtful significance.

Lateral nuclei. The pars anterior (Bodian) is difficult to define in this specimen; a number of scattered cells are found in the place it should occupy. The nucleus lateralis A is degenerated nearly to the same extent as in P. 21, but contains rather more normal cells. Nucleus lateralis B and nucleus lateralis posterior (pars lateralis) are completely degenerated. No other changes were found in the thalamus of this specimen, which in most features is very similar to P. 21. The main points of difference are: the lack of degeneration in the dorsomedial nucleus and in the anterior extremity of the lateral geniculate body; the less complete degeneration of the nucleus anterodorsalis and the nucleus lateralis A. The condition of the lateral geniculate body has already been described by Packer (1941) and need receive no further consideration here.

Experiment P. 4

Survival period: 102 days. The dorsolateral aspect of one hemisphere was exposed widely. After stimulation of the motor area most of the cortex behind it was destroyed with the diathermy. The frontal pole is intact, and also a narrow strip of cortex above the rhinal fissure, and projection fibres in the anterior third of the hemisphere, connected with cortex dorsomedial to the lesion, are unaffected. The posterior part of the lesion is deep, extending through to the ventricle; it encroaches a little on the temporal region, but rather more of the occipital cortex is spared than in either P. 21 or P. 16. There is no damage to the corpus striatum.

Thalamic degeneration

Anterior nuclei. Degeneration is present in the nucleus anterodorsalis and is best seen anteriorly. The anteroventral nucleus is completely degenerated in its posterior third. The anteromedial nucleus appears to contain rather fewer cells than on the normal side, but this finding cannot be considered significant.

Ventral nuclei. The nucleus ventralis anterior shows considerable gliosis and probably some loss of cells; these changes are most marked in the lateral two-thirds of the nucleus. Degeneration is nearly complete in the nucleus ventralis intermedius, but as usual does not extend into its most medial part. In the nucleus ventralis posterior the pars arcuata is mainly affected; few if any neurones can be distinguished in the massive gliosis which has occurred in it. The pars externa is also degenerated, but contains a fair number of normal nerve cells.

Geniculate bodies. The lateral geniculate body is completely degenerated in its posterior two-thirds. The medial geniculate body shows slight but definite loss of cells and gliosis in the central region of its anterior third. The posterior two-thirds are normal.

Lateral nuclei. The pars anterior is normal. The nucleus reticularis shows shrinkage both in total bulk and in individual cells, and there may be some loss of cells. The nucleus lateralis A shows slight gliosis and a definite but incomplete loss of cells, chiefly in its ventroposterior part. Nucleus lateralis B is completely degenerated. The nucleus lateralis posterior (lateral part) is also degenerated, but only in its posterior two-thirds where it is related to the degenerate part of the lateral geniculate body.

Experiment P. 3

Survival period: 92 days. The cortex was exposed from the dorsal aspect and, after stimulation, widely destroyed with the diathermy. The lesion (Fig. 1) as a whole is rather smaller than in the preceding specimens, leaving more cortex intact on the lateral aspect of the hemisphere. The frontal pole is intact, a strip of cortex about 3 mm. wide above the anterior part of the rhinal fissure, the postero-inferior parietal region, all but the most dorsal part of the occipital and the whole of the temporal regions. There is no striatal damage.

Thalamic degeneration

Anterior nuclei. Doubtful signs of degeneration are seen in the anterodorsal nucleus in the shape of slight gliosis and a possible reduction in cell content. The anteroventral nucleus shows considerable loss of cells and gliosis anteromedially; posterolaterally it is normal. Degeneration is complete in the anteromedial nucleus.

Dorsomedial nucleus. This nucleus appears slightly smaller as a whole than on the normal side, but no other evidence of degeneration was found.

Ventral nuclei. All the ventral nuclei are completely degenerated except for the pars medialis (Pl. 3, fig. 12) and the pars arcuata. The latter stands out very clearly in contrast to the other parts of the nucleus.

Geniculate bodies. The lateral geniculate body is completely degenerated in its posterior half. The medial is normal.

Lateral nuclei. The pars anterior and the nucleus lateralis A are normal. The nucleus lateralis B shows definite but incomplete degeneration chiefly in its anterior part. The nucleus lateralis posterior (lateral part) is degenerated, but only posteriorly where it is related to the degenerate part of the lateral geniculate body. The nucleus reticularis again shows marked gliosis, and possibly some shrinkage of nerve cells. There is no clear evidence that any of the latter have been lost. No evidence of degeneration was seen in any other part of the thalamus.

The most important features of this specimen are as follows: complete degeneration in the anteromedial nucleus and degeneration in the anteroventral nucleus which is limited to its anteromedial part (in both these points P. 3 is the converse of P. 4); complete degeneration in all parts of the ventral nucleus with the exception of the pars arcuata and the pars medialis; the absence of significant degeneration from nucleus lateralis A.

The next four specimens (Fig. 2, P. 11, P. 2, P. 5 and P. 6) all have fairly large lesions affecting the motor area and parietal region of the cortex. They are concerned chiefly with the projection of the lateral and ventral nuclei of the thalamus.

Experiment P. 11

Survival period: 159 days. The skull was opened anteriorly and an attempt made to remove the anterior pole of the hemisphere by excision. The lesion (Fig. 2) was found to extend further posteriorly than had been intended, and the frontal pole had not been entirely destroyed. The whole of the motor cortex had been removed together with much of the white matter beneath it and a little of the cortex behind it. Practically all the projection fibres passing dorsomedial to the lesion are divided. The sections show marked evidence of intracranial infection. The ventricle on the operated

side is distended and partly filled with organized exudate, and the cortex as a whole thinner than on the normal side. There is considerable damage (mainly vascular in origin) to the caudate nucleus and rather less to the lentiform. Many cells are lost in the hippocampus and there is extensive subcortical disorganization in the pyriform lobe.

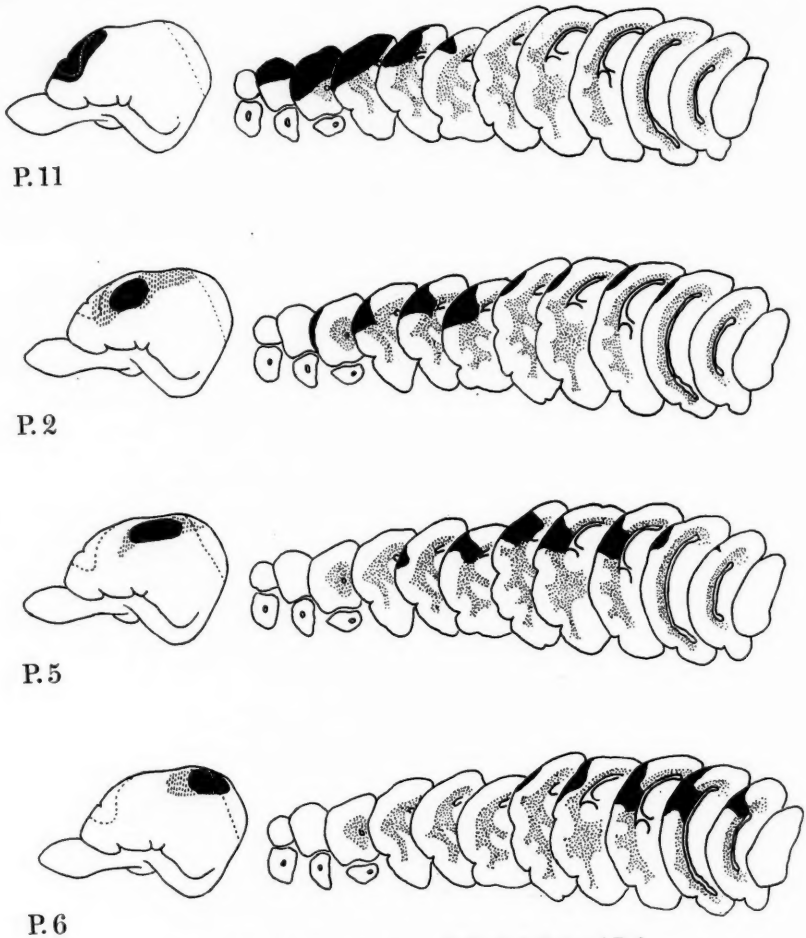

Fig. 2. The lesions in specimens P. 11, P. 2, P. 5 and P. 6.

The widespread nature of the damage caused by infection and secondary vascular changes makes it impossible to place any reliance on degeneration in the thalamus as evidence for projection on to the cortical area destroyed at operation. Negative findings are, however, of some significance, since it can at least be said that thalamic nuclei which remain normal cannot project on to the cortical area actually destroyed. In these circumstances a detailed account of the thalamic degeneration is unnecessary; the principal points are as follows.

In the anterior group the anteroventral nucleus is substantially normal, while the anteromedial nucleus is almost completely disorganized and the anterodorsal only slightly less so. The dorsomedial nucleus shows marked degeneration in its lateral part. Of the ventral nuclei, both parts of the nucleus ventralis posterior are unaffected. Degeneration is marked in the anterior and intermediate ventral nuclei. The nucleus reticularis shows fairly marked degeneration anteriorly. All parts of the lateral nuclei and the geniculate bodies show degenerative changes of a minor character (it is doubtful if there is any actual loss of cells) but no conclusions can be drawn from this observation.

For present purposes the most important feature of this specimen is the absence of degeneration from the nucleus ventralis posterior.

Experiment P. 2

Survival period: 90 days. The parietal region of the cortex was exposed and a lesion made with the diathermy in its anterior part. The principal part of the lesion lies in the anterior parietal region about midway between the dorsomedial border of the hemisphere and the rhinal fissure (Fig. 2).

Thalamic degeneration

In this specimen degeneration is practically confined to the ventral nuclei. The nucleus ventralis anterior shows a partial degeneration. In the nucleus ventralis intermedius degeneration is limited to the lateral half of the nucleus, and, though more marked than in the nucleus ventralis anterior, is still incomplete. The pars externa of the nucleus ventralis posterior shows marked loss of cells and gliosis. A small region in an extreme lateral position is unaffected, and degeneration does not extend posterior to the level of the parafascicular nucleus, so that the posterior pole of the ventral complex is normal. The pars arcuata is substantially normal, although it is possible that a few cells have degenerated anteriorly. The nucleus reticularis shows marked shrinkage and possibly some loss of cells anteriorly, while there is also a great deal of glial proliferation. Only doubtful traces of degeneration were found in any other part of the thalamus.

The most important feature of this specimen is the virtual limitation of degeneration to the ventral nuclei, chiefly the nucleus ventralis intermedius and the pars externa of the nucleus ventralis posterior. It is noteworthy that the pars arcuata is scarcely affected.

Experiment P. 5

Survival period: 91 days. A fairly large piece of cortex was excised from the posterior parietal region. The lesion (Fig. 2) is in the postero-superior parietal region and extends back a little over the border of the visual area. Anteriorly it falls short of the motor area by a considerable distance.

Thalamic degeneration

Anterior nuclei. There is slight gliosis and probably some loss of nerve cells in the dorsolateral and posterior parts of the anteroventral nucleus. The other anterior nuclei are normal.

Lateral nuclei. The pars anterior shows no sign of degeneration, and the nucleus lateralis A is also unaffected to any significant degree. The nucleus lateralis B shows quite marked loss of cells and gliosis anteriorly (Pl. 4, fig. 13); the posterior third of this nucleus is normal. In the nucleus lateralis posterior only doubtful traces of degeneration could be found in the posterior end of its lateral part. The corresponding part of the lateral geniculate body (i.e. about the posterior third) shows similar but more definite signs of degeneration. The nucleus reticularis shows only doubtful cellular changes apart from intense gliosis in the thalamic radiations.

Ventral nuclei. The only undoubted degeneration in these nuclei is in the pars externa of the nucleus ventralis posterior (Pl. 4, fig. 13). Here there is quite definite loss of cells and gliosis in a dorsolateral position adjacent to nucleus lateralis B.

No other degeneration was found in this specimen. Its most important features are the degeneration in nucleus lateralis B and the comparatively slight involvement of the ventral nuclei.

Experiment P. 6

Survival period: 89 days. An area of cortex was excised in the posterior parietal and occipital regions. The lesion (Fig. 2) encroaches on the anterior part of the visual area.

Thalamic degeneration

Degeneration in this specimen is limited to the lateral nuclei and the lateral geniculate body. The latter shows extensive but not quite complete degeneration in all but its anterior third.

Lateral nuclei. The pars anterior and nucleus lateralis A are unaffected. Nucleus lateralis B shows marked but incomplete degeneration mainly localized in its most ventral part adjacent to the ventral nuclei (Pl. 4, fig. 14). The nucleus lateralis posterior shows practically complete degeneration in its lateral part where it is related to the degenerated two-thirds of the lateral geniculate body. No changes other than gliosis are to be seen in the nucleus reticularis.

The most important feature of this specimen lies in the degeneration of nucleus lateralis B, and the lack of degeneration in the ventral nuclei.

The next three specimens (Fig. 3, P. 56, P. 52, P. 54) all have small lesions in the posterior parietal or occipital region, and are relevant to the cortical projection of the ventral and lateral nuclei.

Experiment P. 56

Survival period: 50 days. The lesion (Fig. 3) was made by excision in the lateral aspect of the occipital region. In the sections it was found to lie almost entirely in the peristriate area, but to encroach very slightly on the area striata. The thalamic degeneration is limited to the nucleus lateralis posterior (lateral part) and to the lateral geniculate body. In the lateral geniculate body degeneration is slight. There is little if any loss of cells, but many of those present stain poorly and lamination is less distinct than on the normal side. These changes are confined to the anterior half of the body. In the lateral part of the nucleus lateralis posterior degeneration is more marked than in the lateral geniculate body, but is present only in its anterior half. Here between a half and a third of the normal number of cells has been lost and there is some gliosis.

It is probable that in this specimen the degeneration in the lateral geniculate body is mainly the result of interruption of the optic radiations as they fan out to the posterior part of the area striata.

Experiment P. 52

Survival period: 44 days. A small area of cortex was excised in the superior occipital region. The lesion (Fig. 3) lies in the peristriate area touching the border of the area striata.

This specimen resembles P. 56 very closely, and again degeneration is limited to the nucleus lateralis posterior (lateral part) and to the lateral geniculate body.

The changes in the lateral geniculate body are indefinite: there is slight gliosis mainly in the medial non-laminated portion, but there is no undoubted evidence of

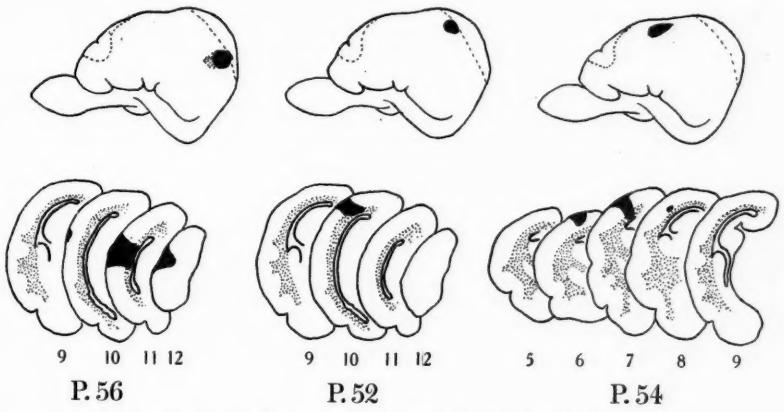

Fig. 3. The lesions in specimens P. 56, P. 52 and P. 54.

loss of cells. In the nucleus lateralis posterior (lateral part) degeneration is more definite: some loss of cells, with degenerative changes in others, and also gliosis. These changes are found only in the posterior half of the lateral geniculate body and the nucleus lateralis posterior, but the last ten sections of both are free from degeneration.

Experiment P. 54

Survival period: 47 days. A small piece of cortex was excised from about the middle of the parietal cortex. The lesion (Fig. 3) is slightly nearer to the area striata than to the motor area. Thalamic degeneration is found only in the nucleus ventralis posterior. It consists of gliosis and an incomplete loss of nerve cells in the lateral half of the pars externa of that nucleus. The nucleus extends through seventy-eight transverse sections ($40\,\mu$) and these changes are found only in the anterior 40. The absence of degeneration from any part of the lateral complex is a striking feature of this specimen.

The next three specimens (Fig. 4) all have comparatively small lesions close to the rhinal fissure.

Experiment P. 57

Survival period: 50 days. The lesion was made by excision a little dorsal to the anterior part of the rhinal fissure. It lies in a highly granular type of cortex between 1 and 2 mm. above the rhinal fissure (Fig. 4). Leading posteriorly from the bottom of the lesion is a neuroglial scar which cuts across the external capsule and ends dorsal to the lentiform nucleus at about the level of the middle of the anterior commissure. This subcortical damage is not great, but has interrupted some fibres which reach the internal capsule after passing through the external capsule and the lentiform nucleus. It is probable that these arise fairly close to the superficial lesion and posterodorsal to it.

Thalamic degeneration is confined to the intermediate and posterior subdivisions of the ventral nucleus. There is a small but definite patch of gliosis and probably some

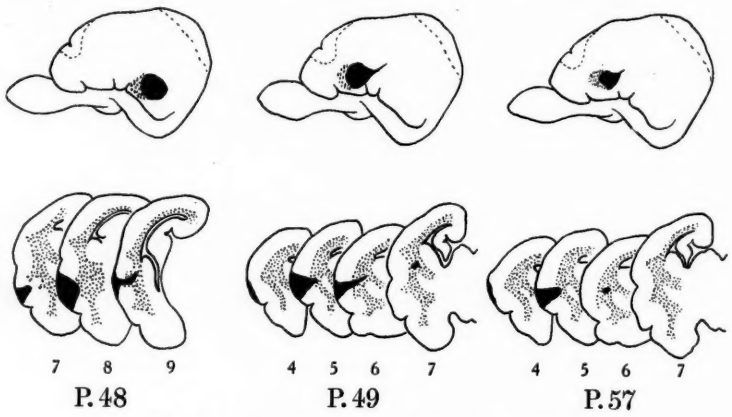

Fig. 4. The lesions in specimens P. 48, P. 49 and P. 57.

loss of nerve cells in the posteromedial part of the nucleus ventralis intermedius. In the nucleus ventralis posterior there is complete degeneration in the medial two-thirds of the pars arcuata (Pl. 4, fig. 15). The pars externa may be slightly affected; it appears to contain slightly fewer nerve cells than normal and slightly more neuroglia.

Experiment P. 49

Survival period: 47 days. A lesion similar to that in P. 57 was made with the diathermy. It was found to lie in the same cortical area (Fig. 4), but to be somewhat larger and to have caused considerably more subcortical damage. It extends in a subcortical position posterodorsally through the external capsule above the lentiform nucleus (which is uninjured) into the internal capsule. In this region it extends posteriorly for some distance and must have divided a considerable number of fibres connected to the parietal and occipital cortical regions. Thalamic degeneration, though more extensive than in P. 57, is confined to the ventral nuclei and the lateral geniculate body.

Nucleus ventralis anterior. There is some loss of cells and gliosis throughout this nucleus.

Nucleus ventralis intermedius. There is slight loss of cells and gliosis in the lateral third of this nucleus.

Nucleus ventralis posterior. Degeneration is practically complete in both parts of this nucleus. It is striking that it does not extend at all into the nucleus laterialis B, but there is slight though definite loss of cells from the posterior part of the lateral geniculate body.

There seems no doubt that in this specimen much of the thalamic degeneration has resulted from the subcortical damage and not from the actual destruction of cortex. Degeneration in the anterior and intermediate subdivisions of the ventral nuclei and in the pars externa of the posterior subdivision is either more or equally marked in some specimens (e.g. P. 3) where the area destroyed in P. 49 was not damaged. The

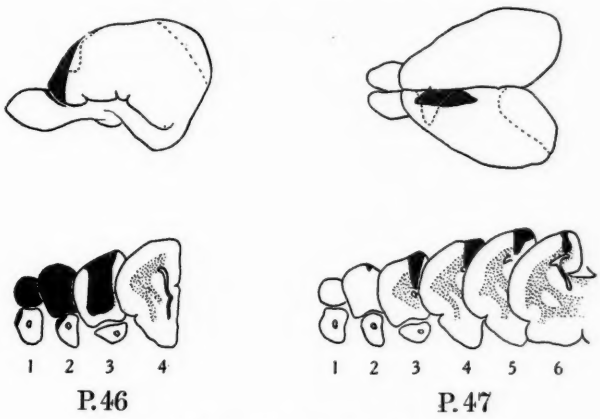

Fig. 5. The lesions in specimens P. 46 and P. 47.

same obviously applies to the lateral geniculate body. This cannot be said however of the pars arcuata of the nucleus ventralis posterior. A survey of the specimens already described will show that this part of the ventral complex degenerates only when the lesion includes or comes very close to the area of cortex destroyed in P. 57 and P. 49.

Experiment P. 48

Survival period: 48 days. The lesion (Fig. 4) was made with the diathermy. It is roughly circular, about 4 mm. in diameter, immediately above the rhinal fissure in the temporal region of the cortex. Thalamic degeneration is practically confined to the medial geniculate body. It is complete (marked loss of cells and gliosis, shrinkage of remaining cells, and general shrinkage in size of the whole body) in all but the caudal pole of the nucleus. Anteriorly the degeneration appears to extend slightly into the ventrolateral part of the nucleus ventralis posterior.

The last two specimens (Fig. 5, P. 46 and P. 47) have fairly small lesions in the anterior part of the hemisphere.

Experiment P. 46

Survival period: 53 days. The frontal pole of the hemisphere was destroyed with the diathermy. The sections show that the lesion extends into the anterior and medial part of the motor area and that, though the frontal pole itself is completely destroyed, the medial, lateral and ventral surfaces of the hemisphere behind it are less affected than the dorsal (Fig. 5). Thalamic degeneration is confined to the dorsomedial nucleus, the condition of which is almost exactly similar to its condition in P. 21, i.e. complete degeneration of the lateral small-celled part of the nucleus, occupying about a third of the transverse area of the whole nucleus anteriorly and about a half posteriorly. No degeneration could be found elsewhere in the thalamus.

Experiment P. 47

Survival period: 52 days. A longitudinal incision was made in the hemisphere close to the dorsomedial border and extending from in front of the motor area into the parietal region. The posterior end of the lesion (Fig. 5) is at the transverse level of the middle of the anterior commissure. It lies about 1·5 mm. lateral to the dorsomedial border of the hemisphere and has divided all subcortical fibres throughout its length. In this way the medial surface of the hemisphere adjacent to the lesion is completely cut off from its projection fibres. In addition a strip of the motor area is destroyed and the destruction extends for a short distance into the cortex behind it.

Thalamic degeneration

Anterior nuclei. The nucleus anteromedialis is completely degenerated, and there is a patch of complete degeneration in the anteromedial part of the anteroventral nucleus (Pl. 3, fig. 11). The anterodorsal nucleus is not affected.

Ventral nuclei. There is a very definite patch of degeneration laterally situated in the pars externa of the nucleus ventralis posterior. This degeneration extends forwards into the lateral part of the nucleus ventralis intermedius. Only doubtful traces of degeneration can be seen in the nucleus ventralis anterior. The pars arcuata of the nucleus ventralis posterior shows no change.

THE CORTICAL PROJECTION OF THE THALAMIC NUCLEI

In this section it is proposed to sum up the evidence for the topographical projection of the thalamic nuclei on the cerebral cortex which can be derived from a study of the specimens described, and to discuss it in comparison with the published descriptions in other animals. In Fig. 6 a general interpretation which is based on this evidence is illustrated. The boundaries which have been marked for the different projection areas are not of course precise; there may well be more overlap than is shown, and the position of some areas has been determined by inference from a number of experiments rather than from a single crucial experiment affecting the area in question alone.

The cortical projection of the thalamus as a whole is illustrated by a specimen such as P. 21, in which almost all the neocortex on one side was removed. In general it may be said that all those thalamic nuclei which, in the phalanger, have been shown to project on to the cortex, have also been shown to have cortical connexions in the other mammals which have been investigated. An exception is Walker's finding

Table 1. *Summary of the thalamic degenerations*

Specimen	Anterior nuclei			Dorso-medial nucleus	Ventral nuclei				Geniculate bodies		Lateral nuclei				Nucleus reticularis
	AD	*AV*	*AM*		*VA*	*VI*	*PE*	*PA*	*LGB*	*MGB*	*LAB*	*LA*	*LB*	*LPL*	
P. 21	++	+++	+++	+++l	+++	+++	+++	+++	+++	?+	+	++	+++	+++	++
P. 16	+a	+++	+++	—	+++	+++	+++	+++	+++	?+	?+	+	++	++	++
P. 4	+a	++p	?+	—	++l	++	++	+++	+++p	+a	—	+vp	++	++p	++al
P. 3	?+	++am	+++	—	+++	+++	+++	—	+++p	—	—	—	+	+p	+
P. 11	++	—	+++	++l	++	++	—	—	+	+	?+	+	+	+	++a
P. 2	—	—	—	—	+	++l	++am	?+a	—	—	—	—	—	—	++a
P. 5	—	+dlp	—	—	?+l	?+l	++dl	—	++p	—	—	?+v	++a	?+p	?+
P. 6	—	—	—	—	—	—	—	—	++p	—	—	—	++v	++p	?+
P. 56	—	—	—	—	—	—	—	—	+a	—	—	—	—	++a	—
P. 52	—	—	—	—	—	—	—	—	?+	—	—	—	—	+p	—
P. 54	—	—	—	—	—	—	++la	—	—	—	—	—	—	—	—
P. 57	—	—	—	—	—	+pm	?+	++m	—	—	—	—	—	—	—
P. 49	—	—	—	—	+	+l	++	++	+p	—	—	—	—	—	—
P. 48	—	—	—	—	—	—	?+	—	—	+++	—	—	—	—	—
P. 46	—	—	—	+++l	—	—	—	—	—	—	—	—	—	—	—
P. 47	—	++am	+++	—	?+	+l	++l	—	—	—	—	—	—	—	?+

The specimens are listed in the order in which they are described in the text and to facilitate comparison have been divided into five groups (for the lesions see Figs. 1–5).

The number of + signs indicates the approximate degree of degeneration in a nucleus, and when unqualified indicates that the whole nucleus is affected; the small letters a, p, d, v, m and l after the + signs show that degeneration is limited to a particular part of a nucleus, e.g. +dlp would mean slight but definite degeneration in the dorsolateral posterior part of a nucleus.

(1938*a*) in the macaque that the nucleus ventralis anterior does not degenerate to any great extent after cortical ablations, unless there is also considerable destruction of the corpus striatum. In at least two specimens in the present series where there was no striatal damage (Fig. 12, P. 3, P. 4), considerable degeneration occurred in the nucleus ventralis anterior. It is, of course, by no means certain that the nucleus so named is directly comparable in two such different mammals as the macaque and the phalanger, but in both it forms the most anterior group of cells in the ventral complex. Clark & Russell (1940) found complete degeneration in the nucleus ventralis anterior in a human case where virtual hemidecortication was effected by a pathological process. Apart from Walker's finding the general statement made above appears to hold, but there are a number of other points which will call for discussion as the individual nuclei are considered in detail.

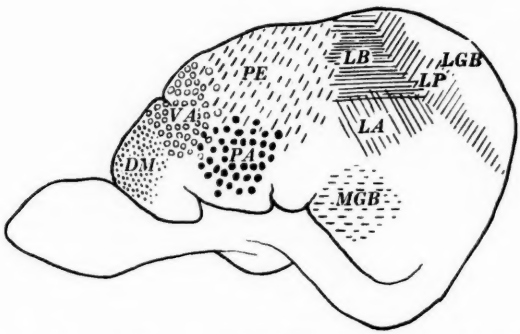

Fig. 6. The projection areas for the different thalamic nuclei on the dorsolateral surface of the hemisphere.

So far as the neocortex is concerned the experiments show that by far the greater part of its area receives projection fibres from the thalamus. It is possible that there are some regions which do not receive such fibres, for example, the posterior temporal and perhaps the inferior occipital regions. This is in accordance with findings in higher mammals, but it is not possible to say more than that none of the results in the phalanger are inconsistent with such an arrangement. A large number of experiments with small lesions and little or no subcortical damage would be required to prove it. It may also be said that, judging from the degree of thalamic degeneration, the ventral nuclei and the geniculate bodies have on the whole more abundant cortical connexions than the lateral nuclei; this is certainly the case when the nucleus ventralis posterior is compared with the nucleus lateralis A or the pars anterior of Bodian; there is less difference between the other ventral and lateral nuclei. The anteroventral and anteromedial nuclei and the part of the dorsomedial nucleus with cortical connexions all degenerate nearly, if not quite, to the same degree as the ventral nuclei, although they usually show less intense gliosis. The anterodorsal nucleus degenerates less completely, but this may be because its projection fibres have not been completely divided in any of the experiments reported.

Throughout the whole series of experiments, certain nuclei have shown no sign of degeneration. In one experiment or another the projection fibres connected with every

part of the neocortex have been divided, except those connected with the posterior temporal and inferior occipital regions, and perhaps with the posterior and inferior part of the cingulate area. It is improbable that any of the nuclei which failed to degenerate are connected with these regions. They have not been shown to have such connexions in other mammals which obviously resemble the phalanger very closely so far as the thalamocortical projection system is concerned. It will be assumed, therefore, that the nuclei about to be considered have no cortical connexions.

The parts of the thalamus which lack corticopetal connexions comprise what is probably a very heterogeneous collection of nuclei. They are generally spoken of as the nuclei of the mid-line and the intralaminar nuclei. Walker (1938*b*) includes in a medial group all nuclei (apart from those of the mid-line) which lie in or medial to the internal medullary lamina, thus putting together such nuclei as the paracentral, the dorsomedial, the medioventral nucleus and so on. Following Clark (1932*a*) the dorsomedial nucleus in the phalanger has been classed as a principal nucleus, and the nucleus which appears to correspond with the medioventral nucleus (see below) lies outside the internal medullary lamina, although it must be admitted that the boundaries of that structure are not very clearly defined. In the present state of our knowledge of the thalamus no classification of these nuclei can be very satisfactory. With a few exceptions such as the parafascicular nucleus, they are not well defined and present the appearance of an undifferentiated matrix from which the cells of the principal nuclei might have been drawn (Papez, 1932). Apart from the centre median nucleus they are all less conspicuous in the thalamus of higher mammals, where the principal nuclei are proportionately larger, and the results of hemidecortication more striking. In man no more than the centre median nucleus and a thin zone of small nerve cells in a subependymal position along the wall of the third ventricle remains to represent them (Clark & Russell, 1940), a very different appearance from that seen in the phalanger or in other primitive mammals (see Pls. 1 and 2, figs. 7–10).

From time to time reports have appeared of degeneration in one or other of these nuclei following a lesion in the cerebral cortex. Such degeneration has usually been the result of hemidecortication where the operative damage has involved at least the corpus striatum as well as the cortex (e.g. Waller, 1938; Bard & Rioch, 1937), or it can be explained as degeneration in the marginal zone of a principal nucleus. No evidence of striatal connexions was found in the present experiments (the corpus striatum was not greatly damaged in any of them), and the evidence for their presence in other mammals is not very satisfactory (cf. Waller, 1938; Clark & Russell, 1940).

The most significant and striking feature of the mid-line and intralaminar nuclei in the phalanger is their size. They form relatively quite a large proportion of the thalamus, a well-recognized primitive characteristic. At the same time the differentiation of individual nuclei is perhaps less marked than in some other mammals. The work of Clark and others has shown that the centre median nucleus is most clearly differentiated in the higher primates; it is represented in the phalanger by cells dorsomedial to the pars arcuata of the nucleus ventralis posterior and related also to the paracentral and parafascicular nuclei, but not clearly defined as a separate element. In a similar situation beneath the paracentral nucleus, but in more anterior parts of the thalamus, other cells probably represent a nucleus submedius which extends forwards into relationship with the nucleus commissuralis interanterodorsalis and the anteromedial nucleus. Again these cells are not clearly defined as a separate nucleus.

What was described (Goldby, 1941) as a posterior extension of the anteromedial nucleus on to the medial side of the nucleus ventralis anterior has also given no evidence of corticopetal connexions. This group of cells clearly corresponds with the nucleus ventralis pars medialis differentiated by Bodian in the opossum (1939), and should be so named in the phalanger. As Bodian points out, a similar group of cells has been described in the rat (Gurdjian, 1927) and in *Tupaia* (Clark, 1929*a*), and a comparable nucleus is present in the cat (Ingram, Hannett & Ranson, 1932) and in primates (Clark & Boggon, 1935; Walker, 1938*a*). There is some evidence for cortical connexions from this nucleus in the rat and cat (Waller, 1934, 1938); Walker found none in the chimpanzee or the macaque (1938*a*, *b*), though Clark & Boggon (1935) showed that part of the nucleus ventralis pars anteromedialis projects on to the cortex. On the whole the evidence for cortical connexions from this nucleus in some animals is a little stronger than for the intralaminar and mid-line nuclei, but by no means unequivocal.

In the remaining parts of the thalamus of the phalanger, the only regions from which corticopetal connexions appear to be completely absent are the medial and ventral parts of the dorsomedial nucleus, and the medial subdivision of the nucleus lateralis posterior. So far as the dorsomedial nucleus is concerned, this finding is in complete agreement with the findings in other mammals (e.g. in the cat and rat, Clark & Boggon, 1933*b*; the macaque, Clark & Boggon, 1935; Walker, 1938*a*). It is noteworthy that the part of the dorsomedial nucleus which lacks corticopetal connexions is large in the phalanger, but is reduced to a very inconspicuous remnant in man (Clark & Russell, 1940).

The medial subdivision of the nucleus lateralis posterior of the phalanger is less easy to identify in other mammals. The nucleus lateralis posterior of lower mammals is probably, in general, comparable with the pulvinar of higher mammals. When the pulvinar as such is well developed, it shows what is described as complete degeneration following hemidecortication (in the cat, Waller, 1938; in the macaque and chimpanzee, Walker, 1938*a*, *b*; and in man, Clark & Russell, 1940). It is relevant to point out that Walker found in the chimpanzee and in the macaque a thin layer of darkly staining cells persisting along the medial margin of the pulvinar after hemidecortication. It may be that this is a remnant of a medial subdivision of the pulvinar (or of the nucleus lateralis posterior of lower mammals) with no corticopetal connexions, which has disappeared in man. Clark & Northfield (1937) found no cortical connexions for a medially placed element, pa, in the pulvinar of the macaque, but in the absence of complete hemidecortication negative evidence cannot be taken as conclusive.

Anterior nuclei. The anterodorsal nucleus shows definite degeneration only in the first group of experiments in which very large cortical lesions had been inflicted (the positive findings in P. 11 are excluded from consideration on account of the damage to the brain caused by infection). Degeneration is extensive only in the hemidecorticate specimen (P. 21); it is definite but not extensive in P. 16 and P. 4, and doubtful in P. 3. The dorsomedial cortex of the hemisphere is affected almost as much in P. 3 as in P. 21, and the chief difference between these specimens lies in the preservation of a considerable amount of cortex ventrolaterally situated, together with much of the cortex on both the lateral and medial surfaces of the occipital region in P. 3. Other experiments show that the anterodorsal nucleus cannot project on to the ventrolateral cortex in the anterior two-thirds of the hemisphere (P. 46, P. 48, P. 49, P. 57), leaving

only the posterior temporal, and the lateral or medial occipital as possible regions on to which it could project. Waller (1934, 1938) in the rat and the cat has produced evidence that the anterodorsal nucleus projects on to the retrosplenial or caudal part of the cingulate area, which accords with Clark's observation (1929*b*) that there is in some animals a relationship between the size of the nucleus in question and the extent of the retrosplenial and cingular areas of the cortex. In the light of what has been said above, it is probable that a similar condition obtains in the phalanger; fibres to the posterior part of the cingulate cortex pass more or less horizontally from the lateral to the medial surface in the occipital region. Such fibres are almost completely spared in P. 3, though considerably damaged in P. 21. It is possible that some of the most ventral fibres have escaped in P. 21, which would account for the incomplete degeneration even in this specimen. Some of these fibres must have been divided in P. 56, with a lateral occipital lesion. The lesion in this case is small, and apparently the subcortical damage is not enough to cause recognizable degeneration in the nucleus.

The anteroventral nucleus degenerates completely after hemidecortication (P. 21), and no degeneration is seen in any specimen unless a considerable number of fibres connected with the dorsomedial cortex of the hemisphere are severed. Though by themselves these observations are not enough to localize the projection area of this nucleus, taken in conjunction with the finding in other mammals that this nucleus projects on to the cingulate gyrus, they justify the conclusion that the projection in the phalanger is similar.

Degeneration is complete only in P. 21 and P. 16, both specimens with very extensive cortical destruction. In others (P. 3, P. 4, P. 5, P. 47) degeneration is present, but limited to definite parts of the nucleus. In P. 3 and P. 47, where subcortical damage is either confined to the anterior region of the hemisphere or most marked there, it is limited to the anteromedial part of the nucleus; in P. 4 and P. 5, where the damage is most marked posteriorly, to the posterior part of the nucleus. These findings suggest a projection over a considerable extent of the cingulate area, with further localization for parts of the nucleus within that area. There are, however, some discrepant findings. No degeneration was seen in the nucleus in P. 2, P. 6 and P. 11, though in each of them fibres connected with the dorsomedial cortex are severed. There is, of course, some difficulty in assessing the precise amount of subcortical damage in any specimen, though its general character may be quite clear, and it is possible that in these particular specimens fewer fibres had been damaged than appeared to be the case, and not enough to cause significant changes in the nucleus.

The anteromedial nucleus, provided the term is restricted to a region with corticopetal connexions, is smaller than it appeared to be in normal material. Judging from the evidence of P. 47, it projects on to a comparatively small area on the anteromedial surface of the hemisphere. This area does not extend on to the medial side of the extreme frontal pole (which is destroyed in P. 46), nor to any considerable extent behind the level of the anterior commissure, since the nucleus is only doubtfully affected in P. 4 and not at all in P. 2, P. 5 and P. 6, where the lesions are in the parieto-occipital region.

These findings agree with those of Clark & Boggon (1933*a*) in the cat and rat, and with Waller's findings (1934, 1938) in the same animals. Walker (1938*a*) found that in the macaque the anteromedial nucleus projects on to the inferior and medial surface

of the frontal lobe. In the absence of more precise homologies than can at present be instituted between the cortical areas of higher and lower mammals, and also of more exact localization of cortical projection areas in the smaller brains, this cannot be considered a discordant finding.

The conclusions, so far as they affect the anterior nuclei, may be summarized as follows. The anterior nuclei in the phalanger project on to the medial surface of the hemisphere, probably to the cingulate area. Within this area the anteromedial nucleus projects anteriorly, the anterodorsal nucleus posteriorly, and the anteroventral nucleus over a more widely spread intermediate region. These conclusions are based on inference from lesions none of which affect the medial surface of the hemisphere directly. Such indirect evidence is not entirely satisfactory, and there are some discrepant findings. It is justifiable to base conclusions on it only because it points in the same way as more direct evidence obtained in other mammals. It is probable that the projection of the anterior nuclei does not differ significantly in the marsupial and placental mammals.

The dorsomedial nucleus. Little remains to be said about this nucleus. From the evidence of P. 46 it is clear that its dorsolateral part projects on to the frontal pole of the hemisphere. It is probable that this projection does not spread into the motor area, since the nucleus is undegenerated in P. 47. So far as it goes this is in agreement with the results in other mammals, e.g. in the cat and rat (Clark & Boggon, 1933*b*), the macaque (Clark & Boggon, 1935; Walker, 1938*a*). In its normal histological features and in its cortical projection, the dorsolateral part of the dorsomedial nucleus corresponds with the pars principalis or the pars parvocellularis in other mammals. Though quite well developed in the phalanger, it forms a smaller proportion of the whole nucleus than in higher mammals.

The ventrolateral nuclei. The nucleus reticularis is considered here because of its close relation to this group of nuclei, and particularly to the pars anterior (Bodian), but it is doubtful if it should be classified with the thalamic nuclei at all. In general it may be said that the nucleus reticularis shows changes which are roughly proportional to the size of the cortical lesion and to the amount of glial proliferation in the thalamic radiations. Changes were found in the first eight specimens described (see Figs. 1, 2), in all of which the lesions are comparatively large; they were least marked and perhaps doubtful in P. 5 and P. 6. No significant changes were seen in any of the other specimens with small lesions with the exception of P. 47, where they were again doubtful. The changes consist of shrinkage of the cells (which stain very deeply), and perhaps in some cases actual loss of cells, but it is difficult to be certain of this. Gliosis is always very marked in the degenerated areas, and it may be that changes in the neurones are due to the local reaction in the thalamic radiations and not to interference with any direct cortical connexions. It seems unlikely that a cell would remain normal if surrounded by such intense tissue reaction as is present in the degenerated thalamic radiations, so that, while the possibility that cortical connexions for the nucleus reticularis may be present, cannot be denied, these experiments cannot be said to establish their presence.

The evidence concerning the pars anterior of Bodian is also unsatisfactory. In the only specimen in which it shows marked degenerative changes (P. 21) these are of the same general type as in the nucleus reticularis, but accompanied by less glial reaction. Only doubtful signs of degeneration were seen in one other specimen, P. 16. In these

circumstances little can be added to what has already been said about this group of cells (Goldby, 1941). The view that it is an outlying group belonging to the nucleus reticularis, rather than a representative of the nucleus lateralis A of higher mammals as Bodian suggests, was then adopted, but was not definitely established. There is a third possibility, that it does not represent the nucleus lateralis A as a whole, but is a group of cells separated in most specimens from the larger nucleus lateralis A of this description by a fascicle of the superior thalamic radiations, but having no morphological importance as an independent nucleus. This would be in accord with its variable character, and also with the fact that it shows degeneration only in specimens in which degeneration is also present throughout nucleus lateralis A, and to a similar degree. This is perhaps the most acceptable suggestion.

The nucleus lateralis A shows significant degeneration only in three specimens (P. 21, P. 16, P. 4), in all of which the lesion is extensive and includes practically the whole parietal region. In P. 3, though the lesion is also extensive, a considerable part of the inferior parietal cortex is spared, and no degeneration is seen in the nucleus. In the remaining specimens, all lesions inferior in position to the lesion in P. 3 lay very close to the rhinal fissure, and the nucleus lateralis A did not degenerate. It is therefore possible that the nucleus projects on to cortex which is intermediate in position between these lesions and the lesion in P. 3, i.e. in the inferior parietal region.

Even in P. 21 the degree of degeneration in nucleus lateralis A is less than in other affected nuclei. It is difficult to say if this is the result of a less abundant cortical projection, or of the fact that the lesion is not a complete hemidecortication. Since slight changes are seen in the medial geniculate body, it is probable that at least the whole of the parietal cortex is destroyed, the lesion extending slightly into the temporal cortex. For this reason it is probable that the specimen P. 21 shows maximal degeneration in the nucleus lateralis A. In this connexion it should be noted that Waller (1938) concludes that in the cat the lateral nucleus, pars anterior, is connected to the cortex but less strongly than some other thalamic nuclei; Clark & Boggon (1935) also found that in the macaque the nucleus lateralis A has either no connexions to the frontal and parietal lobes or very scattered ones, though Walker (1938*a*) finds a localized projection for the corresponding nucleus on the superior lip of the Sylvian fissure. This last finding gives some support to the conclusion regarding the location of its cortical projection in the phalanger, although, in view of the difficulty in comparing the subdivisions of a thalamic nucleus in two very widely separated animals, not much dependence can be placed on it. Clark (1932*b*), using the Marchi method in the rat, traced a connexion to the nucleus lateralis, pars principalis (which from his description appears to correspond with the nucleus lateralis A of the phalanger) from the most dorsal part of the parietal region of the cortex. While admitting the defects in the evidence, it seems reasonable to conclude provisionally that the nucleus lateralis A projects on to the inferior parietal region of the cortex in the phalanger, but that its cortical connexions are less abundant than for most thalamic nuclei; and that in these features it is similar to at least some placental mammals.

When affected the nucleus lateralis B shows more marked degeneration than the nucleus lateralis A. It is almost completely degenerated in P. 21, P. 16 and P. 4, in which practically the whole parietal area of the cortex is destroyed as well as other areas. It is also degenerated (but less completely) in P. 3 where the inferior parietal cortex is largely intact, and in P. 5 and P. 6 where the lesions are limited to the

superior parietal cortex. It is not affected in P. 2 where the lesion lies mainly in the parietal cortex immediately behind the motor area. In P. 54, with a small lesion in the parietal region about mid-way between the motor and the visual areas, there is degeneration in the ventral nuclei but none in the lateral nuclei. The position of the lesion in this specimen may be taken to mark approximately the anterior limit of projection for the lateral nuclei, since P. 6, in which the lesion is confined to the posterior-superior parietal region, shows degeneration in the lateral but not in the ventral nuclei (Pl. 4, fig. 14). P. 52, with a small lesion corresponding in position with the more posterior part of the lesion in P. 6, gave degeneration only in the nucleus lateralis posterior, so that the lesion in this specimen must lie posterior to the limit of the projection area of nucleus lateralis B. This area must, therefore, occupy a comparatively narrow part of the parietal region between the lesions in P. 54 and P. 52, i.e. the posterior parietal cortex, anterior to the peristriate area. Its boundaries mediolaterally have not been determined.

Partly because of differences in terminology, it is difficult to make precise comparisons between the projection of the nucleus lateralis B in the phalanger and in other mammals. It appears to correspond at least in part with the nucleus so named in the macaque (Clark & Boggon, 1935), the hinder part of which was found to project on to the parietal cortex behind the area post-centralis, that is, to the 'parietal association area'. The condition in the phalanger is clearly very similar. So far as the cat and the rat are concerned it is not possible to say more than that there is evidence from both these animals that the lateral complex projects on to the same region of the cortex, but not enough evidence for a more detailed comparison of the projection of the subdivisions of the complex (Clark, 1932*b*; Waller, 1934; Waller & Barris, 1937).

The nucleus lateralis posterior is divisible into a medial large-celled and a lateral small-celled part. The lack of corticopetal fibres for the medial part has already been discussed. The lateral part shows degeneration in all specimens with large lesions involving the posterior parieto-occipital regions of the cortex, and also definite (but more limited) degeneration after small lesions at the border of the area striata (P. 56, P. 52). There is no doubt, therefore, that this part of the nucleus lateralis posterior projects on to the peristriate area of cortex. Two specimens previously reported (Packer, 1941) with lesions within the area striata gave no degeneration in this nucleus; its projection does not, therefore, spread to any great extent into the area striata.

There is evidence that the cortical projection of the nucleus lateralis posterior is similar in the rat (Clark, 1932*b*; Waller, 1934). In the cat the pulvinar degenerates after lesions in the caudal half of the middle supra-sylvian gyrus (Waller & Barris, 1937), and parts of the pulvinar in the macaque project on to the peristriate area (Clark & Northfield, 1937). Allowing for the greater development of the pulvinar in higher mammals and the consequent elaboration of its projection system, these results are consistent throughout the mammalian class.

The findings which concern the lateral complex of nuclei may be summarized as follows. The anterior elements of the complex (the pars anterior of Bodian and nucleus lateralis A) have less abundant cortical connexions than the more posterior elements, and are probably related to the inferior parietal region of the cortex. The nucleus lateralis B projects on to the posterior parietal region in a limited area, which probably corresponds with the parietal association area of other mammals, and the nucleus lateralis posterior (lateral part) on to the peristriate area behind it. Although it is

difficult and perhaps impossible to compare the subdivisions of this nuclear complex in detail from one animal to another, at least when widely different mammals are under consideration, the general uniformity in the cortical projection of the complex as a whole is very m rked. The equivalence of the nucleus lateralis posterior in lower mammals and the pulvinar in higher is reasonably certain, and it is noteworthy that, in the phalanger at least, a large medial part of this nucleus has no corticopetal connexions. This medial part has dwindled to insignificant proportions in monkeys and apes (Walker, 1938 *a*, *b*) and has disappeared altogether in man (Clark & Russell, 1940).

The ventral nuclei in general have abundant cortical connexions, since they show very marked cell loss in retrograde degeneration which is accompanied by more intense gliosis than in any other part of the thalamus. The absence of cortical connexions in the most medial part of the ventral region of the thalamus has already been discussed; in this situation a nucleus ventralis, pars medialis, is found, and also cells from which such nuclei as the nucleus submedius and the centre median nucleus may be developed in other mammals. There remain for consideration the anterior and intermediate ventral nuclei and the pars externa and pars arcuata of the posterior ventral nucleus, all of which are connected to the cortex.

The total area of cortical projection for these nuclei is relatively large. It probably includes the motor area anteriorly (but not the cortex in front of it as is shown by the specimen, P. 46) and extends posteriorly to the mid-parietal region. The posterior border lies about or just behind the level of the lesion in P. 54. It extends ventrally below the motor cortex nearly to the rhinal fissure (cf. P. 57).

The localization of the different subdivisions of the ventral complex within this area is not very clear, but the following tentative conclusions may be drawn. The anterior and intermediate ventral nuclei can be taken together, since with one exception in all specimens in which either is degenerated, the other is equally affected. Such degeneration is seen in the first four specimens (see Fig. 1), in P. 2, P. 47 and P. 49. In all these specimens the motor area and the cortex immediately behind it, or the projection fibres connected with these regions, are injured. This suggests that the intermediate and anterior ventral nuclei project on the anterior part of the total area which receives fibres from the ventral complex, probably including the motor area. Some support is given by the fact that, in P. 54, where the lesion is situated far back in the parietal region, degeneration is limited to the nucleus ventralis posterior. It is unfortunate that more weight cannot be given to the positive evidence of P. 11 (in which much damage was caused by infection) which also supports the above suggestion. If present, projection fibres from the anterior ventral nucleus to the motor area are probably not abundant; in P. 46, where some damage was caused to this area, there was no degeneration in any ventral nucleus, and in P. 47, where the damage to the motor area is considerable, degeneration is not very extensive. In one specimen (P. 57) degeneration is seen in the posteromedial part of the nucleus ventralis intermedius and none in the nucleus ventralis anterior. Since, in this specimen, the lesion is situated close to the rhinal fissure, it suggests that the projection area for the nucleus ventralis intermedius extends farther ventrally than that for the nucleus ventralis anterior.

It is doubtful if the nucleus ventralis anterior and intermedius can be compared exactly with elements in the thalamus of placental mammals, but they certainly correspond with the anterior region of the ventral complex. In a general way their projection is similar to that of the ventral anterior nucleus in the rat (Waller, 1934)

and the cat (Waller & Barris, 1937), and the same general similarity is found in the macaque for the nucleus ventralis anterolateralis and medialis (Clark & Boggon, 1935) and the nucleus ventralis lateralis (Walker, 1938*a*). The absence of cortical connexions from the nucleus ventralis anterior as described by Walker (1938*a*) for the macaque has already been noted.

The nucleus ventralis posterior consists of the two well-marked subdivisions, pars externa and pars arcuata. It is probable that the cortical projection fibres of both are abundant, since after quite small lesions definite and extensive degeneration occurs. The projection of the pars externa spreads farther back in the parietal region than that of any other part of the ventral complex. It is the only region of the thalamus to show degeneration in P. 54, in which the lesion marks the approximate posterior limit of its projection. In P. 11, where the motor area and a little of the cortex behind it is destroyed, no degeneration occurred in either subdivision of the nucleus ventralis posterior; at the same time, the anterior limit of its projection area cannot fall very far behind that for the other parts of the ventral complex as is shown by P. 47. In this specimen the lesion extends only for a short distance into the antero-superior parietal region, but the nucleus shows quite definite degeneration. Inferiorly its projection area falls short by a considerable distance of the rhinal fissure. The inferior limit is probably marked by the superior border of the lesions in P. 49 and P. 57 (Fig. 4). The degeneration which occurred in the pars externa of P. 49 is due to the interruption of subcortical fibres, since the very similar lesion in P. 57 led only to doubtful traces of degeneration in that nucleus. There is some evidence that the superior parts of the cortex are represented in the lateral parts of the nucleus and the inferior in the medial; e.g. in P. 47, P. 54, in both of which the lesion is in the superior parietal region, degeneration occurred in the lateral part of the nucleus, and in P. 2, where the lesion extends further inferiorly, in the medial part.

The pars arcuata of the nucleus ventralis posterior is a particularly well-defined nucleus. In the four specimens with extensive cortical ablations (Fig. 1), it showed complete degeneration in all but one, P. 3. It failed to degenerate in any other specimen except P. 2 (where the changes were slight), P. 57 and P. 49. In P. 49 degeneration is almost complete, and only slightly less so in P. 57. Judging from the last two specimens, it appears that this nucleus projects on to the highly granular cortex above the anterior part of the rhinal fissure a little behind and below the motor cortex. The evidence of these specimens is slightly impaired by the coexistence of subcortical damage, but is supported by the fact that one difference between P. 3 (in which the nucleus remained normal) and P. 21, P. 16 and P. 4 (in which it showed complete degeneration) is that the lesion in P. 3 falls short of the rhinal fissure by a wider strip of cortex than in the other three specimens. A discrepant finding is the degeneration in this nucleus in P. 2, where the lesion does not come very close to the rhinal fissure. The degeneration is slight, and confined to the anterior part of the nucleus where its boundary in a transverse series is difficult to define; it cannot therefore be taken to invalidate the conclusion based on the other specimens. Judging from the intensity of the degeneration, and from the fact that after quite small lesions it involves the whole or the greater part of the nucleus (Pl. 4, fig. 15), the cortical projection fibres are more strictly localized and more closely packed than for most parts of the thalamus.

In comparing these findings with those in other mammals, one again meets the

difficulty that parts of the ventral complex similarly named may not be strictly homologous in different species. In general, however, the posterior part of the ventral complex appears to show medial and lateral subdivisions, and there seems no reason to doubt that the similar subdivisions in the nucleus ventralis posterior of the phalanger are comparable. If so the pars externa is represented by the pars ventrolateralis of Gurdjian (1927) in the rat, the pars externa in carnivora (Rioch, 1929) and in the macaque (Clark & Boggon, 1935), a region which has been further subdivided in the macaque (Walker, 1938*a*) into the nucleus ventralis posterolateralis, postero-inferior and intermedius; the pars arcuata is represented by the nucleus similarly named in other mammals (Waller, 1934, in the rat; Rioch, 1929, in carnivora; Clark, 1937, in the macaque, etc.), or the nucleus ventralis posteromedialis (Walker, 1938*a*, in the macaque). It must again be stressed that, in spite of these general similarities, exact comparison reveals differences which are not unimportant, though their significance may be at present obscure. It has been found impossible, for example, to find any element in the placental thalamus exactly comparable with the nucleus ventralis intermedius as described for the phalanger. It may be that part at least of this intermediate subdivision is included in the posterior subdivisions of other authors.

There is evidence that the ventrolateral part of the nucleus ventralis posterior projects predominantly or exclusively on to the general sensory cortex, e.g. in the postcentral gyrus of the macaque (Clark, 1937), and that the lateral parts of the nucleus are represented in the superior, the medial in the inferior parts of the cortex, and nearer to the rhinal fissure. In the phalanger the projection area for this nucleus extends farther into the parietal region than in higher mammals, and this is probably a result of the poor relative development of the parietal association area.

The projection of the arcuate part of the nucleus ventralis posterior to a region of the cortex a little above the anterior third of the rhinal fissure is fairly definitely established in the phalanger. This region lies very close to that part of the motor area from which movements of the head and neck can be elicited (Goldby, 1939) and may overlap it; movements of the vibrissae can be obtained over a particularly wide area in this region. In the macaque, Clark (1937) has found that the arcuate nucleus projects on to the lowest part of the precentral gyrus, which in that animal is concerned with sensation from the tongue and mouth, and also that it probably receives ascending fibres from the medullary nucleus of the Vth cranial nerve. Walker (1938*a*) has used a different terminology, but his findings appear to be essentially similar. The Vth nerve is well developed in the phalanger, and sensation from the vibrissae plays an important part in the activities of the animal. If the central connexions are similar to those which have been demonstrated in the monkey, this might account for the very definite development of the arcuate part of the nucleus ventralis posterior, and for its abundant connexions to an area of cortex which is, in general, comparable to the cortical area which receives the same fibres in the monkey.

It seems likely that the system of connexions from the medullary nucleus of the Vth nerve to the arcuate nucleus, and thence to an area of cortex close to the motor area for the head and concerned with sensation from the same part of the body, is a stable feature of the mammalian brain, but detailed work on a number of different species is needed to confirm this suggestion. It should be noted that Waller (1934), in the rat, though he finds a similar projection area for the whole posterior part of the ventral complex, finds also that the ventrolateral subdivision projects on to the

antero-inferior parietal and insular regions, and the arcuate nucleus on to the postero-superior parietal region. This is, of course, the converse of the arrangement found here for the phalanger.

The geniculate bodies. The projection of the lateral geniculate body to the cortex has already been described (Packer, 1941), and none of the present findings are incompatible with the conclusions which were then drawn. The geniculocortical projection system appears to be essentially similar in the phalanger and in other mammals.

The medial geniculate body has been shown to project on to the temporal region of the cortex (cf. P. 48). While generally similar to other mammals in this respect, the results do not give enough detailed information for further comparison.

CONCLUSIONS

This experimental investigation has done little more than confirm and in some respects amplify the general conclusions already drawn from an examination of the normal thalamus, viz. that the thalamic structure of the phalanger does not differ fundamentally from other mammals, marsupial or placental, and could be described as typical for a primitive mammal, though showing a number of progressive features. A survey of the literature on the thalamus reveals a fair number of differences, mostly in detail, between different species of mammal, including the marsupials. Owing to the imprecise nature of the methods of investigation it is difficult to assess their importance, and it must be remembered that all the disadvantages of the methods are exaggerated in the examination of the smaller brains of primitive mammals. Partly for this reason, and partly because they have received more attention, knowledge of the thalamus in the larger brains of the higher primates is far more precise and detailed than in any other mammalian Order. A great deal of detailed work on the primitive brains is necessary before it will be possible to stress more than general features of resemblance, or general evolutionary trends affecting such structures as the pulvinar and the proportionate development of whole groups of thalamic nuclei.

The main points in which the conclusions based on a study of the normal thalamus are amplified or modified by the experimental findings are as follows.

The mid-line, intralaminar and other nuclei with no corticopetal connexions are shown to constitute a larger proportion of the thalamus than had been expected. The absence of cortical connexions from a large medial part of the nucleus lateralis posterior is particularly noteworthy, and the presence of cells, also independent of the cortex, medial to the ventral nuclei, and extending from the anteromedial nucleus back to the parafascicular nucleus. It is suggested that these cells represent the nucleus submedius and the centre median nucleus of higher mammals; in the phalanger the only clear nuclear differentiation in them is the nucleus ventralis pars medialis, formerly included in the anteromedial nucleus. In general these features emphasize the primitive rather than the progressive nature of the thalamus.

In the anterior group of nuclei it is found that the anteromedial nucleus is smaller than previously described, provided the term is limited to cells with corticopetal connexions. In view of the poor differentiation and small size of the anteromedial nucleus in the higher primates, this might be looked on as a progressive feature; there is not, however, enough experimental evidence bearing on the connexions of this nucleus in primitive mammals to establish it as such.

The progressive nature of the dorsomedial nucleus, shown by the development of a dorsolateral small-celled part connected to the prefrontal cortex, is confirmed. The degree of difference between the phalanger and other primitive mammals in this respect needs further investigation; it may not be very great.

In the ventrolateral complex of nuclei it is shown that the ventral nuclei have far more abundant and extensive cortical connexions than the lateral; for the lateral nuclei the cortical projection area is quite small and mainly situated in the posterior parietal region. This is again a primitive feature. No additional evidence to support the classification of this complex in a number of different subdivisions has been obtained. In the lateral group it is found that the anterior elements (pars anterior of Bodian and nucleus lateralis A) have fewer cortical connexions than the posterior, and it is possible that the same should be said of the ventral group. Both the nucleus ventralis posterior and the nucleus lateralis posterior are more obviously comparable with similar subdivisions in other mammals than the anterior or intermediate subdivisions. The apparent greater stability of these parts of the complex may be a result of their relation to the lemniscus systems on the one hand, and to the visual system on the other. In particular the comparison between the medial and lateral parts of the nucleus ventralis posterior and similar subdivisions in other mammals would appear to be valid. The clear differentiation of the medial subdivision is very striking, and evidence has been produced which at least suggests strongly that it projects on to a sensory area for the head, in turn making it likely that it receives fibres from the trigeminal lemniscus as the pars arcuata probably does in primates. There is not enough evidence to say whether these are progressive features, or whether they are features common to all mammals but showing minor variations from species to species.

Though the experimental findings do not support the validity of differentiating such nuclei as the nucleus ventralis anterior or intermedius, or the nucleus lateralis B, they do not suggest that such differentiation has no significance. In view, however, of the difficulty of allowing for the personal factor in defining nuclei whose boundaries are by no means clear, little importance can be attached to their comparative significance. Grünthal (1934), in describing the thalamus of another marsupial (*Didelphys aurita*), which from his photographs is clearly of the same general type as that of the phalanger, has used a terminology and a subdivision of the principal nuclei which is radically different from that used by Bodian for *Didelphys virginiana*, or here for the phalanger. Even in the same animal agreement is not always reached as the descriptions for the thalamic nuclei given by Clark & Boggon (1935) and Walker (1938*a*) in the macaque show.

It is in the lateral geniculate body that the most unequivocal evidence of progressive trends is found. This evidence has already received adequate discussion (Goldby, 1941; Packer, 1941). What has been found for the medial geniculate body adds nothing to this.

It is clear that, taking all features together, the primitive are far more striking than the progressive. The latter owe their main significance to the fact that they are so obviously parallel to progressive features which have developed to a higher degree in placental mammals, and show no evidence for the appearance of divergent evolutionary trends or specializations in the marsupial brain. One may conclude that all the fundamental characteristics of the mammalian thalamus were present, actually or potentially, in the common ancestors of the marsupial and placental mammals.

SUMMARY

The corticopetal connexions of the thalamus of *Trichosurus vulpecula* have been investigated by the method of retrograde cell degeneration following cortical injuries which have varied from almost complete hemidecortication to small lesions 2–3 mm. in diameter. The following points have been established:

The anterior nuclei project on to the cingulate area (the evidence for this is inferential since none of the lesions involved this area directly), the anteromedial nucleus on to the medial side of the frontal lobe, the anteroventral nucleus posterior to this and above the cerebral commissures, the anterodorsal nucleus possibly to the posterior cingulate cortex. The evidence for the localization of the projection of the anterodorsal nucleus is less satisfactory than for the other two nuclei, but it certainly has cortical connexions.

The ventral complex of nuclei projects on to a wide area including the motor area and the anterior half of the parietal cortex, and extending ventrally almost if not quite to the rhinal fissure. The nucleus ventralis anterior has cortical connexions probably with the motor area and the cortex immediately adjacent; no separately defined area has been found so far for the nucleus ventralis intermedius; the nucleus ventralis posterior, pars externa, projects on to the anterior parietal cortex and the pars arcuata on to an area immediately above the anterior part of the rhinal fissure, probably a sensory area for the head.

The medial geniculate body projects on to an area which corresponds with the auditory area in other mammals; the details of the projection of the lateral geniculate body have previously been reported (Packer, 1941).

The lateral complex of nuclei projects on to the posterior parietal region of the cortex. The nucleus lateralis A has comparatively few cortical connexions, probably with the ventrolateral part of this region. The nucleus lateralis B projects dorsomedially in the post-parietal region and the nucleus lateralis posterior (pars lateralis) on to the peristriate area.

No evidence of corticopetal connexions has been found for the nuclei of the mid-line, the intralaminar nuclei, the parataenial and parafascicular nuclei, the nucleus ventralis pars medialis or the nucleus lateralis posterior (pars medialis). A centre median nucleus is probably present, but is not clearly defined; it has no corticopetal connexions.

While showing a few progressive features, the thalamus and its corticopetal projection system in *Trichosurus vulpecula* are very similar to those of other primitive mammals; no features which could be described as specifically marsupial were found.

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ABBREVIATIONS USED IN THE FIGURES

AM	nucleus anteromedialis.
AV	nucleus anteroventralis.
CL	nucleus centralis lateralis.
DM	nucleus dorsomedialis.
LA	nucleus lateralis A.
LAB	nucleus lateralis, pars anterior (Bodian).
LB	nucleus lateralis B.
LGB	lateral geniculate body (nucleus dorsalis).
LP	nucleus lateralis posterior.
LPL	nucleus lateralis posterior, pars lateralis.
LPM	nucleus lateralis posterior, pars medialis.
MGB	medial geniculate body.
PA	pars arcuata of nucleus ventralis posterior.
PE	pars externa of nucleus ventralis posterior.
VA	nucleus ventralis anterior.
VI	nucleus ventralis intermedius.
VM	nucleus ventralis, pars medialis.
VP	nucleus ventralis posterior.
VPA	nucleus ventralis posterior, pars arcuata.
VPE	nucleus ventralis posterior, pars externa.

EXPLANATION OF PLATES 1–4

The illustrations are all untouched photographs of transverse sections of the thalamus, cut in celloidin at 40μ and stained with toluidine blue.

PLATE 1

Fig. 7. Section showing the anterior nuclei in P. 21. Complete degeneration is seen in the nucleus anteroventralis and anteromedialis, with particularly marked gliosis in the former. Degeneration is incomplete in the nucleus anterodorsalis, but the contrast with the normal side is quite obvious; the large size of its commissural nucleus is well seen. All nuclei dorsomedial to the internal medullary lamina are normal. General shrinkage in size on the affected side is very marked. Magnification $\times 20$.

Fig. 8. Specimen P. 21, twelve sections posterior to the section in Fig. 7. The principal lateral, ventral and dorsomedial nuclei have all appeared. There is a small patch of degeneration outlined in the nucleus dorsomedialis; some normal cells are seen near the superficial aspect of the nucleus lateralis A; the nucleus ventralis anterior is completely degenerated. The nucleus ventromedialis is clearly seen and completely normal. Magnification $\times 20$.

PLATE 2

Fig. 9. Specimen P. 21, six sections posterior to Fig. 8, showing nucleus lateralis A, nucleus ventralis intermedius, etc. Degeneration is well marked in the dorsomedial nucleus. The nucleus lateralis, pars anterior (Bodian), can be seen and shows little degeneration; degeneration in the nucleus ventralis intermedius does not extend medial to the mammillothalamic tract. Magnification $\times 20$.

Fig. 10. Specimen P. 21, forty-six sections posterior to Fig. 9. Complete degeneration is seen in the nucleus ventralis posterior, in the nucleus lateralis posterior (pars lateralis), and in the lateral geniculate body. The normal ventral nucleus of the lateral geniculate body and pars medialis of the nucleus lateralis posterior are clearly seen. The nucleus parafascicularis is also normal. Magnification $\times 20$.

PLATE 3

Fig. 11. Specimen P. 47, anterior nuclei. Complete degeneration is seen in the nucleus anteromedialis and in the medial part of the nucleus anteroventralis. Magnification ×20.

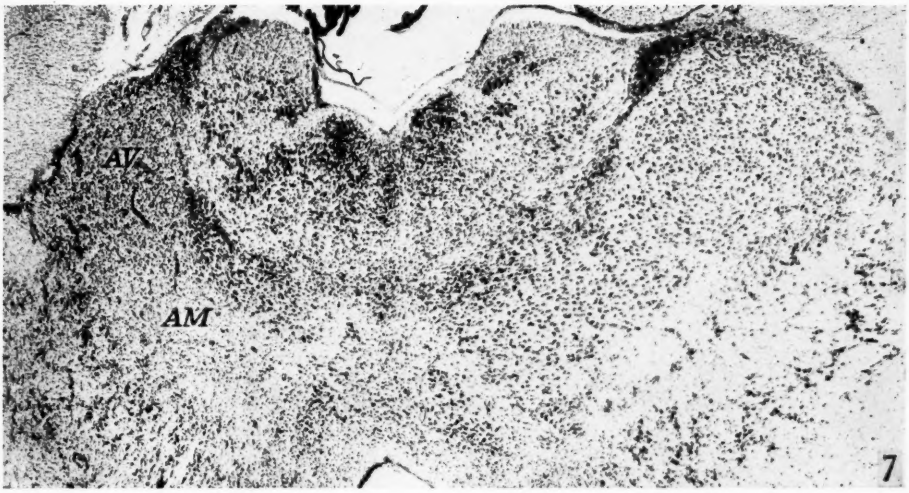
Fig. 12. Specimen P. 3, showing complete degeneration in the nucleus ventralis anterior. Magnification ×20.

PLATE 4

Fig. 13. Specimen P. 5. The section passes through about the middle of the thalamus. Complete degeneration is seen in the nucleus lateralis B with unusually marked gliosis. The degeneration extends into the pars externa of the nucleus ventralis posterior. Magnification ×20.

Fig. 14. Specimen P. 6. This section corresponds in level to the section of P. 5 in Fig. 13. Degeneration is confined to the nucleus lateralis B. Magnification ×20.

Fig. 15. Specimen P. 57. The section, which corresponds in level to the sections in Figs. 13 and 14, shows degeneration in the pars arcuata of the nucleus ventralis posterior. There are no significant changes in the pars externa. Magnification ×50.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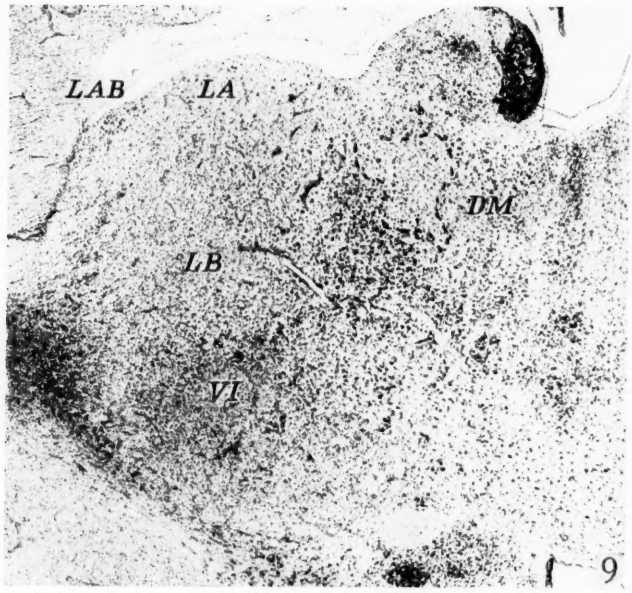
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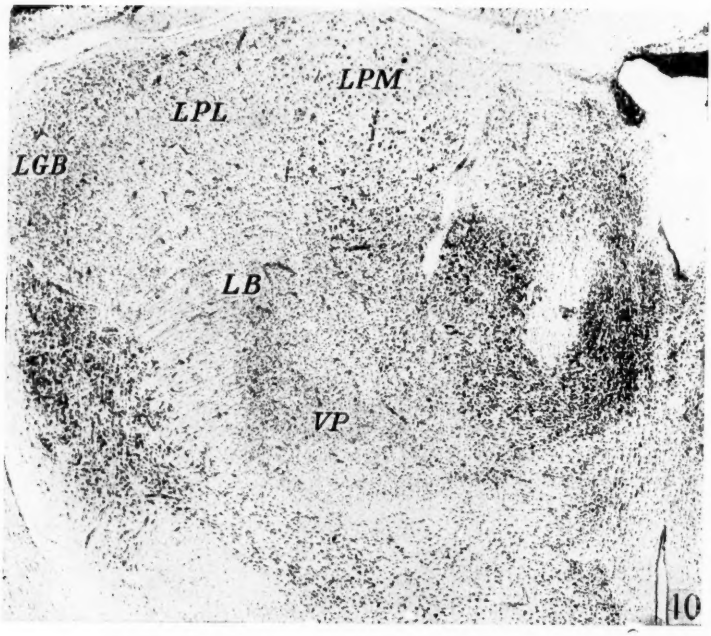
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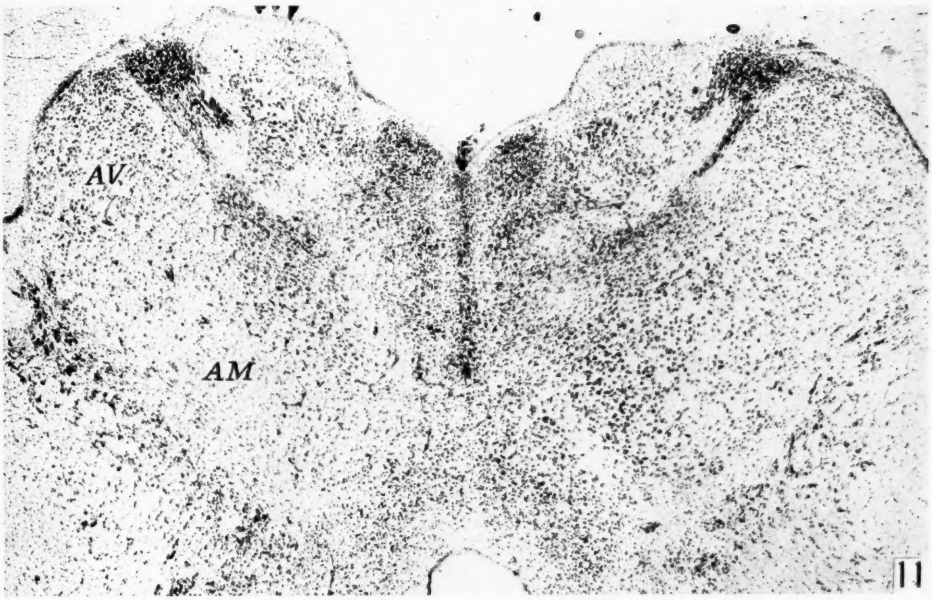
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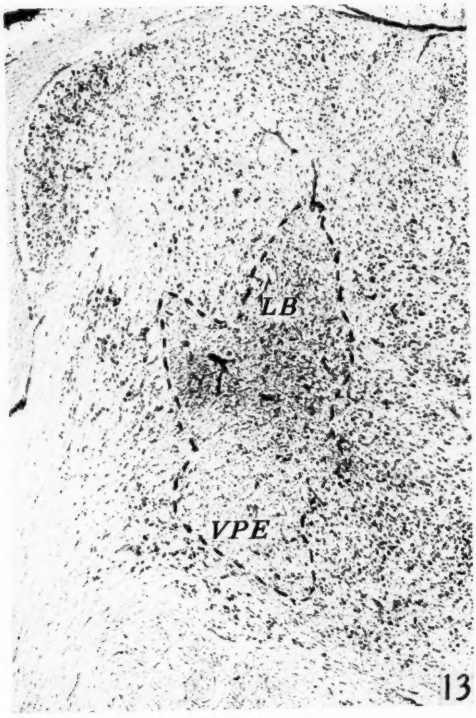
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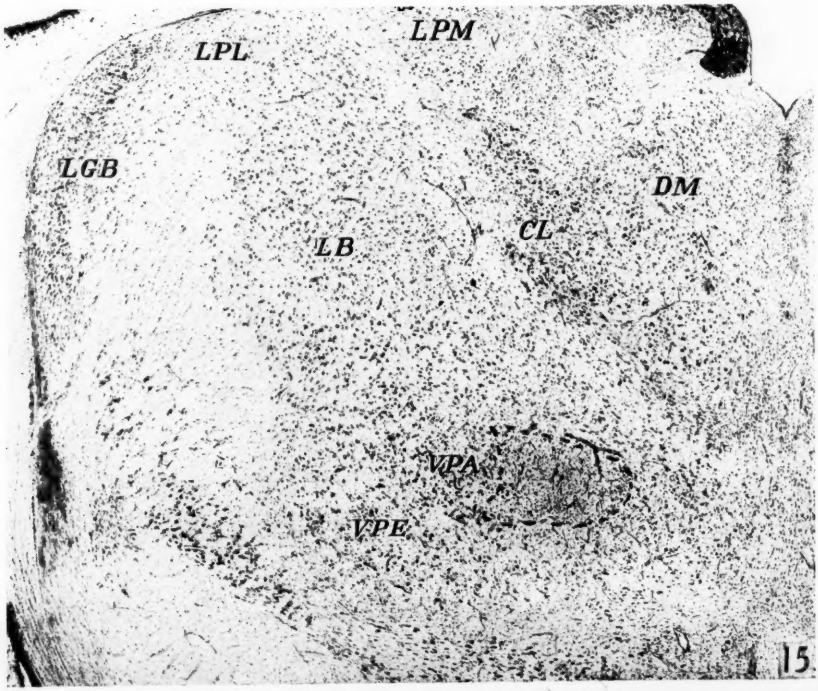
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THE DEVELOPMENT OF THE CONJUNCTIVAL PAPILLAE AND OF THE SCLERAL BONES IN THE EMBRYO CHICK

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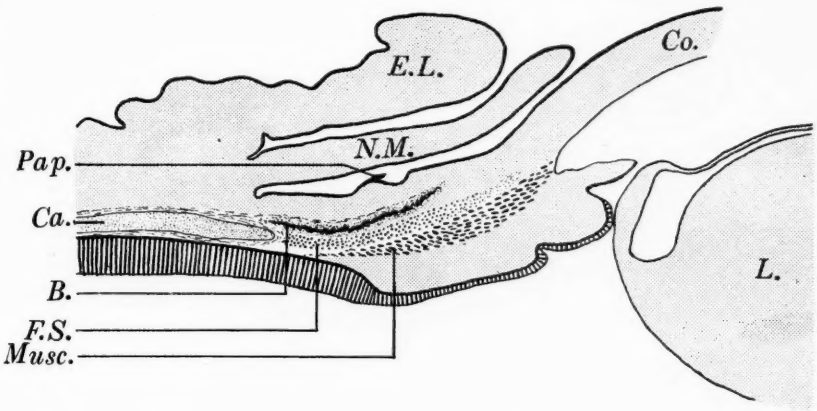
It is well known that a series of fourteen epidermal papillae is present on the conjunctiva of the chick embryo between the seventh and fourteenth days, arranged in a ring round the pupil. Somewhat later, from about 10 days, the fourteen scleral bones begin to appear, one developing in the mesenchyme beneath each epidermal papilla. The development and fate of the papillae have been described by Nussbaum (1901). His account, though in large part confirmed by Dabelow (1927), is in important respects erroneous, and a new description is necessary. The present paper contains a description of the development and fate of the papillae, of the early development of the scleral bones, and of a structural relation, previously described by me in *Nature* (1941), between each papilla and the bone developing beneath it.



Text-fig. 1. A diagram showing the position of the epidermal papillae and scleral bones with reference to other structures. *B.* bone; *Ca.* scleral cartilage; *Co.* cornea; *E.L.* eyelid; *F.S.* fibrous sclera; *L.* lens; *Musc.* ciliary muscle; *N.M.* nictitating membrane; *Pap.* epidermal papilla.

Nussbaum found that the papillae appear at about 6 days of incubation and that at 13 days they are only recognizable microscopically, while at hatching no trace of them remains. This accords with my own observations.

Text-fig. 1 is a diagram, based on a section of a 12-day embryo, showing the topographical relation between the papillae, the bones and the neighbouring parts of the eye. The scleral bone lies between the cartilage and the cornea, overlapping the cartilage peripherally. Above it, the papilla is shown rising from the epidermis, and below it is indicated a ring of fibrous tissue which extends from the cartilaginous part of the

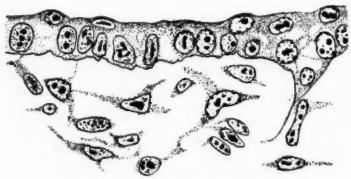
sclera into the cornea. It will be referred to as the 'fibrous sclera'. Beneath this again is the ciliary muscle.

MATERIAL

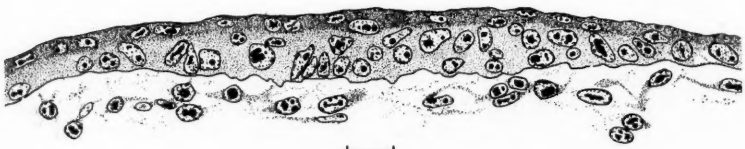
I have studied the following material:

5-day embryo: one eye;
6-day embryos: one eye from each of three embryos;
7-day embryos: one eye from each of five embryos;
8-day embryos: one eye from each of two embryos;
9-day embryos: both eyes from two embryos;
10-day embryos: one eye from each of two embryos;
12-, 17-, 19-day embryos: one eye each.

Fixation was in some cases with Zenker, in others with Susa; the stain was in all cases Azan, except for a few slides from the 19-day embryo which were stained with haematoxylin and eosin.



Text-fig. 2. Unmodified conjunctival epidermis and some of the mesenchyme beneath it. 7 days. Azan. Camera lucida.



Text-fig. 3. A papilla early in stage 1, with a little of the mesenchyme beneath it. 7 days. Azan. Camera lucida.

OBSERVATIONS

No papillae were found in the 5- and 6-day embryos; this agrees with Nussbaum and Dabelow.

The development and regression of the papillae can be arbitrarily but conveniently divided into six stages.

Stage 1 (Text-figs. 2, 3, 9A)

Early stages in the development of the papillae can be found at 7 days. At this time the unspecialized epidermis between the papillae has the structure shown in Text-fig. 2. It is a two-layered epithelium, the cells of the lower or basal layer being approximately cubical, while those of the upper layer or periderm tend to be flattened. It retains this structure without important change at least as late as 17 days of incubation.

One of the earliest papillae which I have seen is shown in Text-fig. 3. It is a flat thickening of the epidermis in which the periderm cells appear to be unchanged while the basal layer is two or three nuclei deep, the whole being scarcely twice the thickness of the unaltered epidermis. In slightly more advanced papillae, the periderm becomes two or three cells thick. The order of magnitude of these thickenings may be illustrated from one of them, which could be traced through seventy-four sections 5 μ thick and in this dimension (approximately tangential to the pupil) therefore measured 370 μ. The length of the thickening in the plane of the sections (approximately at right angles to the tangent to the pupil) was about 180 μ.

In this stage mitoses can be found rather sparsely in the upper and lower layers of the epithelium both in the thickened region and elsewhere; but in the thickened region very nearly all, if not all, are in the peripheral part of the thickening and not near its centre. The axis of the mitoses is usually horizontal or nearly so. The greatest thickness of each papilla is at its centre, to which cells appear to be migrating from its more peripheral parts (see stage 2). The degenerative phenomena which play so important a part in later stages are not seen in stage 1. This statement must be qualified by reference to a single papilla in a 7-day chick, in which the structure of the papilla as a whole is typically that of stage 1, but the whole of the periderm is in active degeneration. This does not usually happen until stage 2.

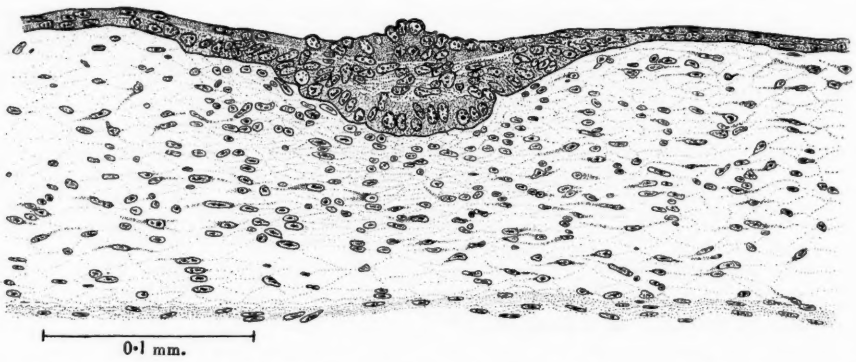
Casual examination gives the impression that the mesenchyme beneath the epidermal thickenings is slightly denser than in neighbouring regions, but an attempt to confirm this by counting nuclei in this region and in the mesenchyme in regions between epidermal thickenings failed to show any difference. An attempt to find a difference in the size of mesenchyme nuclei led to a similar negative result. It seems, therefore, that if there is any condensation in the mesenchyme at this stage it is very slight.

Stage 2 (Text-figs. 4, 9B; Pl. 1, fig. 2)

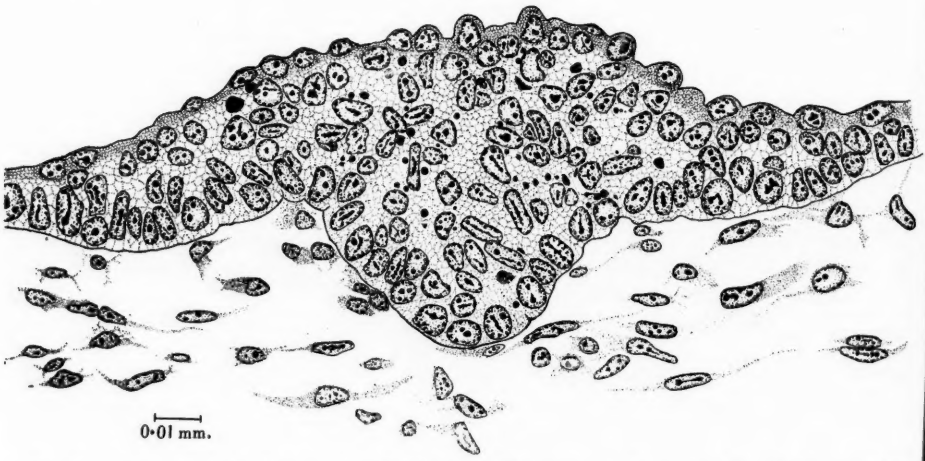
I have found papillae of stage 2 only in 7-day chicks, except for one in an 8-day chick. In this stage there appears a differentiation between the central and peripheral parts of each thickening. The central region increases in thickness, beginning to bulge downwards into the mesenchyme, and, to a less extent, upwards above the general level of the epidermis (Text-fig. 4). The cells responsible for this increase in the thickness of the central region seem, from the histological picture, to have originated as basal layer cells in parts of the thickening a little away from the central region; they appear to migrate out of the lower layer, and then centripetally between the periderm and basal layers, so that they form a 'central mass' which causes the downward bulge. At the same time the basal layer cells covering the bulge become more regularly columnar. When it is first forming, the downward bulge may be of irregular form, consisting of several rounded protuberances.

The periderm may now be two, three or even four nuclei deep and the cells rounded or cubical, not flattened as they are in unmodified parts of the epidermis and in the papillae during stage 1. During stage 2 degenerative changes begin in these cells. Details of these changes resemble those seen on a larger scale in other parts of the papillae in later stages, and some description is given of them under stage 3. The uppermost nuclei tend to project above the general surface of the epithelium as though being extruded, and this probably is the fate of some. These changes are confined to the periderm and to its more central part; in the peripheral parts of the papillae the

cells remain healthy. The occurrence of mitoses is as in stage 1. During stage 2 the mesenchyme underlying the papilla begins to show definite condensation, which is densest close to the downward projection of the central part of the papilla and gradually thins out in all directions (Pl. 2, fig. 1). The cells are more numerous than



Text-fig. 4. A papilla in stage 2, 8 days, showing an early stage in the formation of the 'tongue', the limiting cells of the lower layer becoming columnar, and the papilla beginning to project slightly above the rest of the epidermis. There is a diffuse condensation in the mesenchyme beneath the papilla. Azan. Camera lucida.



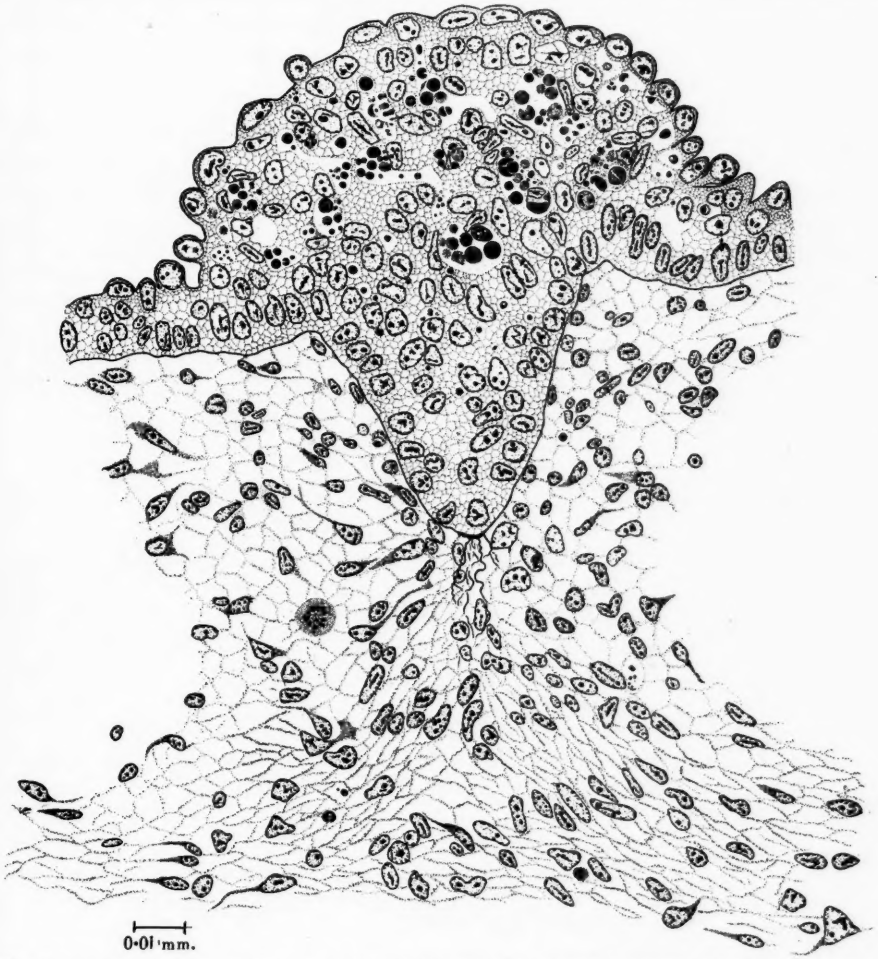
Text-fig. 5. Stage 3, 8 days. Formation of the tongue. The entire papilla except for a single superficial layer of cells is derived from the lower layer of the epidermis. Degeneration granules are appearing. Azan. Camera lucida.

in neighbouring regions, but show with Azan no differentiation of form or structure. They are mostly fusiform with their long axes roughly parallel to the plane of the epidermis. The nuclei vary from oval to elongate, and have one or two nucleoli. The cells lie in a very delicate fibrillar stroma which takes aniline blue feebly, if at all. In contrast with later stages this stroma shows no disturbance beneath the papilla

except in being pushed downwards as the downward projection of the papilla becomes longer.

Stage 3 (Text-figs. 5, 6, 9C; Pl. 1, fig. 3)

This stage, which I found in chicks of 7 and 8 days' incubation, is marked by an increase in the size of the downward projection from the papilla, which becomes a



Text-fig. 6. A more advanced specimen of stage 3, 8 days. There is much degeneration in the central mass of cells at the base of the tongue, with the formation of vacuoles. Slender collagen fibres arising from the tongue run among the cells of the mesenchyme condensation; this is the 'descending strand'. There are a few degenerate cells in the mesenchyme. Azan. Camera lucida.

large conical structure (Text-figs. 5 and 6). It is here called the 'tongue'. At the same time the papilla begins to project more above the general surface of the epidermis. These changes are expressions of the increase of the central part of the papilla, brought

about by the continuation of the centripetal migration of cells mentioned in stage 2. At the height of stage 3 (Text-fig. 6) the papilla consists of (*a*) a superficial epithelium of cells derived from the original periderm, (*b*) on the surface towards the mesenchyme a fairly regular columnar epithelium of basal layer cells, (*c*) the 'central mass' filling the tongue and lying between it and the periderm, derived from the basal layer cells which migrated here from their original positions nearer to the periphery of the papilla.

Mitoses can still be found in the papillae at this stage, but only in their more peripheral parts, and the number varies considerably from papilla to papilla. In five papillae 1, 1, 4, 5 and 8 mitoses respectively were found.

In stage 2 degeneration occurs among the cells of the periderm, but not elsewhere. In stage 3 the periderm cells tend to project above the general surface, the nuclei being in their free ends, but they show less degeneration than in the preceding stage, and less in the more advanced papillae of stage 3 than in the less advanced. Basophil granules may be found in their cytoplasm, but the wave of cellular destruction which is apparent in stage 2 has apparently receded. The degenerating cells have either been cast off, which is strongly suggested by certain papillae of stage 2, or have recovered, which is almost certainly not the case. There is, however, as stage 3 progresses, increasing degeneration in the cells of the central mass. It begins in the base of the tongue or above it, the cells forming the tongue itself being at first less affected. Its onset is marked by the appearance in the cells of basophil granules (staining with azo-carmine). These seem to originate in two ways: in the cytoplasm, which contains granules varying in size from tiny specks hardly visible with an oil-immersion objective to particles larger than nuclei, and from the nuclei themselves. In nuclear degeneration, the nucleus seems first to stain more deeply with azo-carmine and also more diffusely, and then to lose all visible structure as the basophil substance increases. Finally, the nuclei probably become indistinguishable from particles of cytoplasmic origin. At first the basophil granules are found rather sparsely in scattered cells (Text-fig. 5), but the number of affected cells increases, and in the more advanced specimens of stage 3 there are large vacuoles which seem to have bounding membranes and which are filled with basophil detritus (Text-figs. 6, 7). The columnar layer bounding the tongue against the mesenchyme remains unaffected. Its cells are now more regularly columnar than in stage 2.

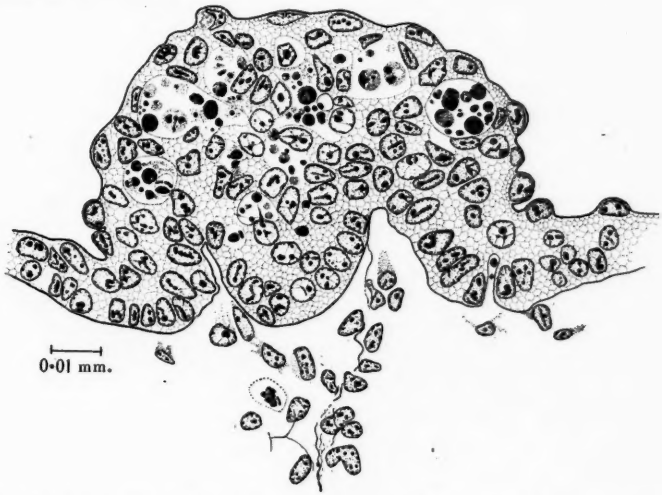
The condensation of the mesenchyme below the papilla is more obvious than in stage 2 (compare Pl. 1, figs. 2, 3). During stage 3 it changes in form and in the arrangement of its cells and fibrous stroma. In stage 2 there is a diffuse condensation whose cells lie with their long axes parallel to the general plane of the conjunctiva (Text-fig. 4; Pl. 1, fig. 2), and the form of the whole condensation is roughly that of a thick disc, the condensation occupying nearly the whole space between its tongue and the fibrous continuation of the sclera. Later, and especially in stage 4, the condensation ceases to be disc-like and arranges itself as a thick column, with its longest dimension at right angles to the plane of the conjunctiva (Text-fig. 6; Pl. 2, fig. 5, a papilla of stage 4), while the cells lie, with their long axes more vertically than horizontally, in a fibrillar stroma which is similarly orientated (Text-fig. 6). A few mitotic figures can be found in the condensed mesenchyme and also occasional degenerating cells (Text-fig. 6).

An important observation at this stage is the presence of fine collagen fibres arising from the basement membrane of the tongue and spreading out among the cells of the

condensation (Text-fig. 6). These fibres are distinct from those forming the stroma of the mesenchyme, for they stain with aniline blue while the stroma scarcely stains at all; they do not form a harmonious part of the stroma, but run among the cells of the mesenchyme without reference to its arrangement. In stage 3 they stain only weakly, though quite decisively, with aniline blue, and are very fine, frequently by no means easy to see; they become a more prominent feature in later stages.

Stage 4 (Text-figs. 7, 9D; Pl. 2, fig. 5)

The principal change in this stage is the regression of the tongue, and this is accompanied by a further increase in the elevation of the papilla above the general surface of the conjunctiva. I have found papillae of stage 4 in 8- and 9-day embryos, and one in a 7-day embryo.



Text-fig. 7. Stage 4, 8 days. The papilla, a few cells of the mesenchyme condensation, and the upper part of the descending collagen strand. The tongue is sinking into the mass of liquefying cells at its base. Azan. Camera lucida.

The disappearance of the tongue is a consequence of the continued degeneration of its cells and of cells forming its foundation in the central part of the papilla. This degeneration, which begins in the central mass at the base of the tongue, spreads into the substance of the tongue itself, so that soon the whole central part of the papilla, except its upper and lower limiting layers, is in active degeneration, and it becomes a spongy mass riddled with large vacuoles (Text-fig. 7). Into this the tongue, reduced by the progressive degeneration and liquefaction of its cells, subsides. The degeneration of the cells which form most of its bulk causes the aspect of the papilla facing the mesenchyme to become more and more concave, except for the still projecting tongue. Finally, the tongue disappears altogether, and the papilla thus becomes a hollow structure whose cavity opens on to the mesenchyme (Pl. 1, fig. 1). This is the beginning of stage 5.

An attempt to count mitoses in six papillae of this stage showed them to be rare.

Three were found in one papilla, two in another, and in the remainder none, a total of five in six papillae as against nineteen in five at stage 3. As in earlier stages, the few found were situated in the peripheral parts of the papillae.

The mesenchyme condensation is still arranged as described in advanced specimens of stage 3 (Text-fig. 6; Pl. 2, fig. 5), as a sort of column extending from the papilla down to the fibrous continuation of the sclera. In the axial part of this column the stroma is particularly dense and its orientation is vertical to the plane of the conjunctiva; from this region it sprays out on either side to become continuous with the general stroma of the connective tissue, which is orientated parallel to the conjunctiva. The descending strands of collagen fibres, which were first detected in stage 3 running from the tongue into the mesenchyme condensation, are now somewhat more prominent and more numerous, and can be traced further into the mesenchyme. Some of them are shown in Text-fig. 7. They run predominantly in the axial zone of the condensation, but spray outwards from it, losing themselves in the more peripheral parts.

In the most advanced specimens of this stage the papilla stands high above the epidermis, but the tongue is so reduced that it no longer projects beyond the concavity into which it has sunk. The mesenchyme condensation is dense, and its form is beginning to change again, for as it is followed downwards below the papilla it begins to spread or flatten outwards. The collagen strands from the tongue run through it spraying out among its cells, but I suspect that, in addition to this, tiny sprigs of collagen have here and there been formed in the mesenchyme as the very first indication of the approaching formation of bone.

Stage 5 (Text-figs. 8, 9E; Pl. 1, fig. 1, Pl. 2, fig. 6)

Papillae in stage 5 were found in 8- and 9-day embryos. Pl. 1, fig. 1 and Pl. 2, fig. 6* show a papilla rather early in this stage. The tongue has disappeared completely, the surviving part of the papilla is elevated higher above the general level of the epidermis than in earlier stages and it is concave below, having a cavity which is widely open to the mesenchyme and is occupied by mesenchyme cells. The cells which originally formed the central mass and the substance of the tongue are greatly reduced in quantity, but some still exist, forming the greater part of the wall of the papilla. Basophil granules and vacuoles show that their degeneration is still going on. From the cavity of the papilla collagen fibres run down and spray out among the cells of the mesenchyme condensation. Later in stage 5 (Text-fig. 8) the form of the papilla changes, becoming more elongate, so as to be still further elevated above the general surface of the conjunctiva, while at the same time it narrows, especially at the base. Its cavity is in its lower half and opens by a small aperture on to the mesenchyme below. The cavity still contains mesenchyme cells, and from it the bundle of collagen fibres runs down into the mesenchyme. The papilla still consists of the surface epithelium of periderm cells, an irregularly cubo-columnar epithelium of basal layer cells lining the cavity, with some degenerate remains of the 'central mass' between the two.

A search for mitoses in twelve papillae showed three in one papilla, one in another, and in the remainder none. The two in which mitoses were found were both young specimens.

* Both these figures show the same section, Pl. 1, fig. 1 being a retouched photograph which is a mirror image of the correctly orientated Pl. 2, fig. 6, an untouched photograph.

In the earlier specimens of this stage the mesenchyme condensation has the vertical orientation and columnar form described in stage 4, but later this changes. The condensation begins to spread outwards, flattening itself above the fibrous sclera. In other words, the columnar condensation of stage 4 becomes disc-like again. This

Text-fig. 8. Stage 5, 9 days. Filiform papilla, still with a cavity. The descending collagen strand is very prominent. Notice the degeneration in the mesenchyme near the descending strand, between the papilla and the young bone, which is not shown. Azan. Camera lucida.

flattening process is not completed in stage 5, and even in the more advanced specimens condensed mesenchyme intervenes between the base of the papilla and the mesenchyme disc; this is not the case in stage 6.

Numbers of collagen fibres, in more advanced specimens staining brilliantly with aniline blue, run from the cavity of the papilla down through the core of mesenchyme, spraying out among its cells (Text-fig. 8; Pl. 1, fig. 1). Mitoses occur in the condensed

mesenchyme. The degenerate cells seen in the mesenchyme condensation in earlier stages are now much more numerous, and are found associated with the descending strands of collagen fibres (Text-fig. 8), but are rare or absent in the flattened part of the condensation.

In the flattened part of the condensation of the more advanced specimens very delicate blue-staining collagen fibrils are now appearing between the cells. These are the beginnings of the collagen fibrils of the scleral bones. Their orientation is in harmony with the pre-existing stroma, which does not stain with aniline blue or only stains extremely feebly, and they are probably formed in part by a thickening of its fibrils. They are not found in any part of the condensation except its basal region, precisely in the position to be occupied by the scleral bone. The much thicker and more striking descending collagen strands coming down from the papilla can be traced right through the condensation to this 'bone' (Pl. 1, fig. 1).

Stage 6 (Pl. 2, fig. 4)

In stage 6, which was found in 9- and 10-day embryos, the final regression of the papillae commences and the developing bone becomes firmly established.

The first change in the papillae is the reduction of the cavity to a narrow canal and then its final disappearance, so that each papilla becomes a solid structure arising from the slightly thickened conjunctiva. The layer of cubo-columnar cells which lined the cavity may for a time persist as a core of nuclei in the axis of the papilla, but their arrangement becomes irregular and soon they can no longer be recognized. The collagen fibres which lay in the cavity can still be found after it has disappeared, penetrating into the base of the papilla (Pl. 2, fig. 4), and running between its cells for varying distances, sometimes through about half the length of the papilla. It frequently ends in a little knot of collagen, in about the position which must earlier have been occupied by the end of the cavity. In younger specimens in which it is still possible to recognize the remains of the cubo-columnar lining of the cavity, the papilla retains its earlier structure, viz. a superficial epithelium covering a mass of degenerating cells between it and the remains of the cubo-columnar layer. Later, the degenerating material seems to disappear, and in more advanced specimens (Pl. 2, fig. 4) the papilla is reduced to a rather flattened filiform structure, two or three cells thick near the base and only one cell thick near the apex, with few or no obvious signs of degeneration such as vacuoles or basophil granules. It seems to consist wholly of periderm cells, all the cells derived from the basal layer having degenerated and disappeared. In length the papillae vary. Some are tiny structures consisting of only a few cells, like that shown in Pl. 2, fig. 4, but in another the length was 135 μ, its width near the base being 76 μ and near the apex 55 μ, so that it has a rather ribbon-like form. The papilla often ends distally in a little vesicle. Degenerative changes can still be found in the area of thickened epidermis from which the papilla proper arises; indeed, it has not hitherto been noticeable in the neighbouring thickened epidermis. Mitoses do not occur in the papilla at this stage, but they do occur in the unmodified epidermis of the conjunctiva.

In the younger specimens of stage 6, although most of the mesenchyme condensation is now flattened out in the position of the future bone and is beginning to ossify, a small cone of condensation still extends upwards to the base of the papilla. In this region the stroma is vertically orientated and the descending strands of collagen fibres

pass downwards in harmony with it; but when the condensation is fully flattened out and becoming ossified, the stroma of the connective tissue between the epidermis and the developing bone is orientated in planes parallel to the epidermis and the descending collagen strands cross it disharmoniously, without reference to its orientation, as shown in Pl. 2, fig. 4.

The descending collagen strand itself emerges from the base of the papilla usually as a single thick fibre which runs downwards, fraying out as it goes into smaller fibres and fibrils, which continue to diverge and branch as they approach the developing bone, with whose fibres they can be seen to be continuous. In the mesenchyme around the descending strand there are usually found, especially in the younger specimens, a number of degenerating mesenchyme cells, but these are less numerous in such advanced specimens as that shown in Pl. 2, fig. 4.

The cells of the bone rudiment itself are at first mesenchymatous in character, with very delicate collagen fibrils between them, but as the fibrils multiply and thicken the cells take on the character of osteoblasts, increasing the quantity of their cytoplasm and becoming irregularly rounded or polyhedral. In the more advanced specimens (Pl. 2, fig. 4) the bone fibres have so increased in number and staining power that a coherent sheet of bone is present, in whose denser parts it becomes difficult to distinguish individual fibres. On both surfaces it is thickly covered with osteoblasts, between which run the delicate new collagen fibres which are being added to the matrix. A few bone cells are already imprisoned in the developing bone, becoming osteocytes. At its end remote from the pupil the bone anlage just overlaps the edge of the scleral cartilage (Text-fig. 1), and here fades out into a layer of connective tissue which extends peripherally just external to the perichondrium of the cartilage. Later this layer of the connective tissue is added to the perichondrium. At its central end (towards the pupil) the bone fades into a slight condensation of mesenchyme into which fine collagen fibres extend from it; this is evidently merely the central end of the original mesenchyme condensation, not yet ossified. The bone has not yet any clearly defined periosteum; on its surface towards the conjunctiva a zone of the mesenchyme is flattened against it, and this is evidently the beginning of the fibrous component of the periosteum. On its opposite surface the same is true, but the bone lies so close against the fibrous sclera that it is not easy to say whether the periosteum should be distinguished from it or not. Later, no such distinction is possible.

Later stages

In an eye of a 12-day embryo only four papillae were found, the remainder had disappeared. A descending collagen strand could be found with certainty in only one of these cases, and in this it could not be traced to the bone. In two of the other cases scraps of collagen were present embedded in the epidermis at the base of the papilla, and a few collagen threads in the mesenchyme were perhaps remains of the descending collagen strands.

The bone is as described in stage 6, but a little further advanced. Some osteoblasts have been included as osteocytes. Since the bone stains with aniline blue and not at all with azo-carmine it is apparently still uncalcified.

At 17 and 19 days no papillae were seen. The bones in these later stages were very much thicker, being about 25 μ at the thickest part by comparison with less than 10 μ at 12 days. Each bone, which is calcified, appears to be a simple plate without cavities

or eminences. With both Azan and haematoxylin and eosin the bone substance appears homogeneous in texture and no attempt was made to study its fibrillar structure; it is not lamellar. Osteoblasts are present on both outer and inner surfaces, and there is an accumulation covering the central edge (near the cornea), and to a less extent the radial edges; on the other hand, at their peripheral ends (i.e. remote from the pupil), the bone ends in connective tissue without osteoblasts. It is evident from this that growth is in thickness and in surface area, and the growth in area is centripetal but not centrifugal. The growth in thickness seems to be more rapid on the lower surface (that towards the retina) than on the upper, for on the upper surface the osteoblasts are more flattened and the surface of the bone smoother, while the lower surface is made irregular by the bays in which lie osteoblasts fixed in the process of becoming osteocytes.

The bones lie between two layers of fibrous periosteum which are common to them all; there is no fibrous periosteum between two bones, and where they overlap one another there is only a loose and delicate connective tissue between the two sets of osteoblasts. On the upper surface the fibrous periosteum continues into the outer layers of the perichondrium, and that on the lower surface is identical with the fibrous sclera.

DISCUSSION

(1) *The work of Nussbaum and Dabelow*

The earlier and more complete of the two antecedent descriptions of the development of the papillae is Nussbaum's. It is difficult to understand how Nussbaum arrived at the views he expresses. With his description of the early condition, when the papillae are flat thickenings (my stage 1), I am in accord, but thereafter, according to him, the papillae develop in two different ways according to whether or not the underlying mesenchyme takes part. Generally, he says, it plays no part, and in these cases the solid epidermal papilla projects both above and below the epidermis. This accords well with my stages 3 and 4. Nussbaum described in detail the degenerative changes in the papilla, but he thought that the degeneration led to the disappearance of all the cells except those lining the tongue against the mesenchyme, and that the result was the formation of a deep crater full of degenerating cells. These are cast off, leaving a flat pit or pocket, lined by epithelium, sinking from the epidermis down into the mesenchyme, and having a lumen which opens on to the surface. I have never seen anything resembling this. In Nussbaum's figures of this stage (his Figs. 12 and 13) the mesenchyme is omitted and reference has to be made to the description of figures to find out which side of the epithelium is supposed to be towards the mesenchyme and which away from it. It is perhaps significant that if the correct orientation of the figures were the reverse of that stated by Nussbaum, they would satisfactorily represent papillae in my stage 5. The second mode of development described by Nussbaum, in which the connective tissue does play a part, he believes to be less common. Short cylinders are formed which extend above the epidermis like feather germs, and they have a cavity containing connective tissue cells. He figures only one papilla in this stage (his Fig. 10); it and the description agree well with my stage 5.

In my material I find no evidence that there are alternative modes of development or that Nussbaum's hollow pockets opening on to the surface exist at all. I have made drawings of over fifty papillae and have studied more, and all that I have

examined fit satisfactorily into the single developmental story outlined in the descriptive section. All Nussbaum's and Dabelow's figures fit too, except Nussbaum's Figs. 12 and 13, which would be more convincing if the mesenchyme were shown.

(2) *Epidermal thickenings and mesenchyme condensations*

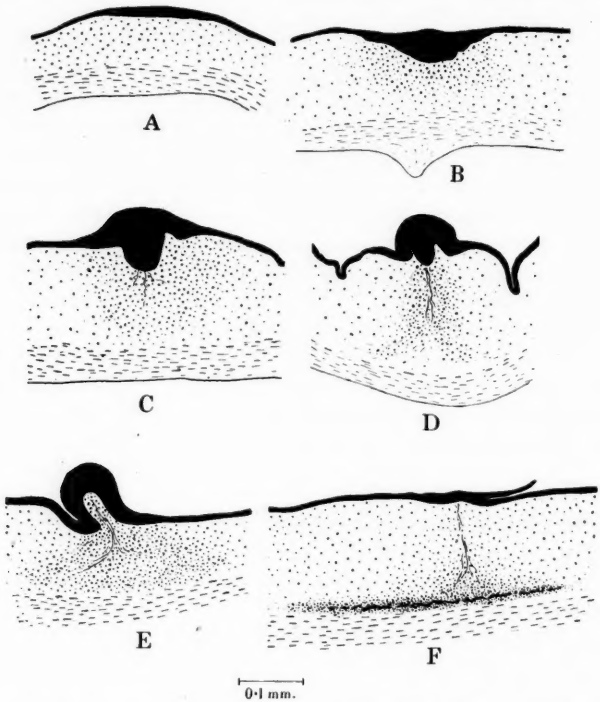
The more interesting observations described in the present paper are: (1) the recognition of the osteogenetic cells first as a mesenchyme condensation, not at the site of their subsequent differentiation, but separated from it by a short distance and associated with a transient epidermal thickening in whose development and fate cellular degeneration plays an important part; (2) the presence of the 'descending strands' of collagen fibres, whose development increases as the cells move towards the site of their differentiation; and (3) the occurrence of degeneration among the osteogenetic cells particularly during this movement. It is interesting to notice similar events in other structures.

In the developing mandible of the chick Jacobson & Fell (1941) found that the osteogenetic cells, which will later form the dermal bones, originate in mesenchymal proliferation centres which lie below two thickenings of the epidermis. Although these thickenings are much smaller and less striking structures than the scleral papillae, the resemblance is sufficient to hint at a probable underlying similarity in the physiology of development. Another instance is probably the epidermal thickening found at the apices of limb-buds in many vertebrate embryos; in these the occurrence of cellular degeneration (Glücksmann, 1934) accentuates the resemblance to the scleral papillae. A third is the association, described by Pehrson (1922), between early stages in the development of certain dermal bones in fish and neuromast organs. Moy-Thomas (1941) found that the dermal bones develop normally even when the formation of neuromast organs is prevented, but Westoll (1941) has suggested that the experiments should be repeated on more primitive forms. The nearly normal development of isolated proximal fragments of limb-buds of 3- and 4-day chicks, which necessarily lack the thickened epidermal cap because this is at the distal end of the limb-bud, similarly seem to deny any significance to the association. Nevertheless, it is difficult to believe that a resemblance between such diverse animals and structures is wholly accidental.

(3) *Cell degeneration and collagen fibres*

Since the works of Glücksmann (1930, 1934), of Fell (1939) and of Jacobson & Fell (1941), there can be no doubt of the importance of cell degeneration as an event in morphogenetic processes. The degeneration here described in the scleral papillae resembles those described by Glücksmann in the epidermal thickening at the tips of limb-buds and by Fell in the mid-ventral epidermal ridge in birds. Degenerative changes in the mesenchyme were reported by Jacobson & Fell in the mandible and by Fell in the avian sternum, in both cases associated with cell migrations. In the present work the degenerating mesenchyme cells were found around the descending strand of collagen fibres, where the mesenchyme migrates downwards to its definitive position, but they are found rarely if at all in early stages before there has been any migration or in the final position where migration has ceased. The collagen strands first appear in stage 3 but become prominent only in stage 4, when the downward migration of the mesenchyme begins, and reach their maximum development in stage 5. Thus there seems to be a connexion between cell degeneration, the formation of strands

of collagen fibres, and the migration of cells. Very similar collagen fibres were described by Fell in her work on the sternum, again in association with migration.



Text-fig. 9. Diagrams summarizing the history of the papillae and of the mesenchyme condensations; all the diagrams are based on actual sections. A, stage 1; B, stage 2; C, stage 3; D, stage 4; E, stage 5; F, stage 6.

SUMMARY

1. The epidermal papillae on the conjunctiva of the embryonic chick are present at 7 days' incubation as flat epidermal thickenings. In 7- and 8-day embryos the central part of each thickening has increased greatly and projects downwards into the mesenchyme as a conical mass (the 'tongue'). The papilla also projects slightly above the general level of the epidermis. Degeneration soon sets in among the epithelial cells of the papilla, especially in and at the base of the tongue, and this part of the papilla liquefies. As a result, the tongue retracts and disappears, while at the same time the elevation of the papilla above the general level of the epidermis increases, and it changes from a solid mass projecting principally downwards into the mesenchyme, into a hollow, more or less filiform structure, projecting upwards from the epidermis. Its cavity is open to the mesenchyme and contains mesenchyme cells. Finally, the papillae lose their cavities and dwindle away; by 12 days most have disappeared.

2. The rudiment of the bone is first recognizable as a diffuse condensation of mesenchyme cells below and around the tongue, separated by a short distance from the site

at which it later differentiates into bone. At about the time when the tongue of the papilla is regressing, the cells of the condensation begin to move downwards, and finally flatten out in the position of the future bone. During the migration collagen fibres appear running from the basement membrane of the papilla, especially in the tongue, among the mesenchyme cells. These fibres are called 'descending collagen strands'. Associated with them are numbers of degenerating mesenchyme cells. After the disappearance of the tongue, when the mesenchymal precursor of the bone has arranged itself as a flat sheet, its cells transform themselves into osteoblasts and the development among them of matted collagen fibrils is the beginning of the bone matrix. The descending collagen strands now run from the papilla to become continuous with the developing collagen of the bone. Degeneration among the mesenchyme cells ceases.

3. In later stages the bone grows in thickness and in surface area. Growth in thickness is principally on the lower surface, growth in area at all the edges except that remote from the pupil; the bone thus grows centripetally but not centrifugally. The whole series of bones is contained between a single upper and a single lower fibrous periosteum. The upper periosteum is continuous with the upper perichondrium of the scleral cartilage, while on the lower side the fibrous periosteum is identical with the fibrous continuation of the sclera towards the pupil. At the end towards the cornea, both layers of periosteum become merged with its fibrous systems. Where the bones overlap one another the two osteoblast layers are separated only by a delicate fibrillar sheath.

It is a pleasure to acknowledge that the expenses of this work were provided by a Government Grant of the Royal Society. I would also like to express my indebtedness to my colleague, Prof. W. J. Hamilton, of the Department of Anatomy in this College, who kindly permitted the use of his photographic apparatus, and to Mr Arthur Westwood who took the photographs.

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EXPLANATION OF PLATES 1 AND 2

Plate 1

Fig. 1. A papilla and underlying mesenchyme early in stage 5, 9 days. The tongue has disappeared and the papilla contains a cavity which is open to the mesenchyme below and contains mesenchyme cells. The descending strands of collagen fibres run from the cavity of the papilla down into the mesenchyme, and towards the bottom right-hand corner of the figure join the delicate fibrils of very early bone. The figure is a retouched photograph. Azan.

Fig. 2. Stage 2, 8 days. A photograph showing the diffuse mesenchyme condensation beneath the papilla, and beneath this again the fibrous sclera. Azan. × 150.

Fig. 3. Stage 3, 8 days. The mesenchyme condensation is more pronounced than in fig. 2. The tongue is more strongly developed. Azan. × 150.

Plate 2

Fig. 4. Stage 6, 10 days. The papilla has lost its cavity. The formation of bone is advanced and the descending collagen strand is traceable from the papilla into the collagen of the bone. The figure was drawn, with a camera lucida, from two adjacent sections. Azan.

Fig. 5. Stage 4, 8 days. The tongue is beginning to regress and the mesenchyme condensation is arranged like a broad column beneath the papilla. The papilla is shown in detail in Text-fig. 7. Azan. × 150.

Fig. 6. Stage 5, 9 days. The mesenchyme condensation is flattening out against the fibrous sclera. The papilla is shown in greater detail in Pl. 1, fig. 1. Azan. × 150.

Fig. 2

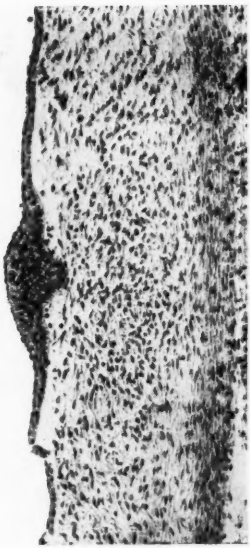

Fig. 3

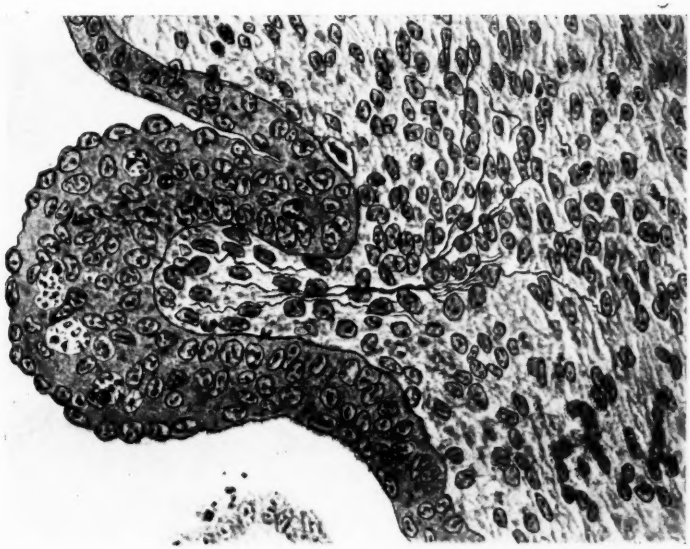

Fig. 1

Fig. 5

Fig. 6

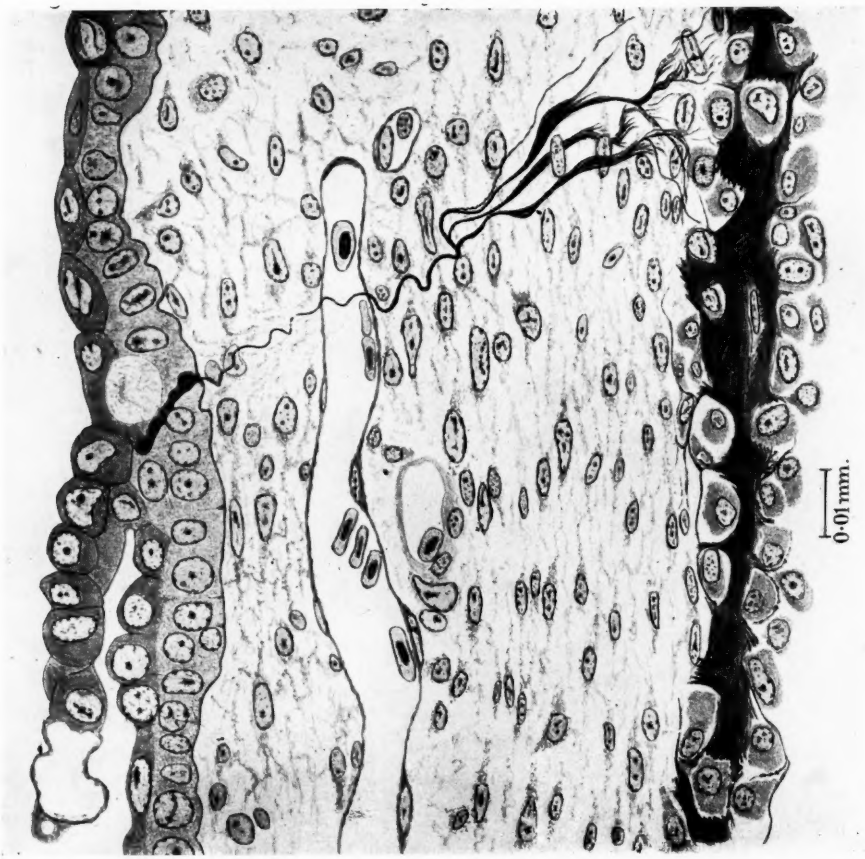

Fig. 4

ON THE PRESENCE OF NERVE CELLS IN THE NEUROHYPOPHYSIS OF THE DOG

By WILLIAM M. SHANKLIN, *Department of Histology, School of Medicine, American University of Beirut*

It is the purpose of this paper to show that typical nerve cells are found in the neurohypophysis of the dog, both in the stalk and in the infundibular process. As a matter of fact, some of these nerve cells are so conspicuous that the writer's attention was directed to this study by the chance observation of a large round nerve cell in a set of serial sections of the neurohypophysis stained by haematoxylin and eosin.

Lothringer (1886) believed the neurohypophysis to be chiefly neuroglial tissue, but prior to that date most histologists were of the opinion that it consisted primarily of connective tissue. Retzius (1894), using the Golgi method, was the first to establish clearly the neuroglial nature of these cells. Berkley (1894), following what he refers to as the ordinary rapid silver impregnation method, described in the neurohypophysis of the dog what he thought were nerve cells with dendrites and axons. Herring (1908), studying the neurohypophysis of the cat stained by Nissl's method, said the cells described by Berkley did not resemble true nerve cells. Stendell (1914) also emphasized (p. 37) that newer staining methods failed to prove these were nerve cells. Bucy (1930), using the Hortega silver carbonate method, stained the finer processes of these cells and confirmed their neuroglial character. However, Bucy noted that they were not identical with astrocytes, oligodendroglia or microglia; hence he called them pituicytes. According to Vazquez-Lopez (1942) some atypical microglia are present in the pars nervosa of the horse. Gersh (1939), Hickey, Hare & Hare (1941) have studied the osmophilic inclusions in the cytoplasm of the pituicytes and Shanklin (1940) their histogenesis.

After the neuroglia, the most abundant element in the neurohypophysis is nerve fibres that descend from the hypothalamus, pass through the stalk and spread out through all the infundibular process. Ramón y Cajal (1902) includes an excellent drawing (Fig. 644) of these fibres. Their exact origin and distribution have been clarified by more recent investigators. Rasmussen (1938) summarizes these findings and includes a full list of references.

A third element in the neurohypophysis is the connective tissue, which has been studied especially by Trossarelli (1934). In addition to neuroglia, nerve fibres and connective tissue, other cells may be found, such as basophils in the human. Mast-like cells have been described in cattle, cat, and man by Gray (1935) and in cattle by Tavares de Sousa (1937).

Authors of textbooks and other current literature rarely refer to the presence or absence of nerve cells in the neurohypophysis and when mentioned their existence is flatly denied.

The material included in this study consisted of the entire hypophyses and the adjacent part of the hypothalamus of ten adult dogs, cut at 8 μ in the sagittal plane. Hypophyses A to H were stained by the Nissl method (methylene blue); hypophyses I and J with haematoxylin and eosin. Each section was examined systematically and the number and location of all nerve cells were recorded.

The general form and the components of the pituitary of the dog are well known. Rioch, Wislocki & O'Leary (1940, pp. 24–28) review the terminology of this region. In order to avoid any complications of terminology the terms infundibular stalk and infundibular process will be used. Comparing their Figs. 21 A (monkey) and 21 B (cat) with that of the dog, it is readily observed that the dog is intermediate, especially regarding the depth to which the third ventricle penetrates into the neural lobe. In the monkey the ventricle terminates well up in the stalk, in the dog very close to the level of the junction of the stalk with the process, but in the cat well down in the infundibular process. In the hypothalamus of the dog, nerve cells are very abundant, but in the region where the anterior limb of the stalk is attached they stop very abruptly. In sagittal sections a band of tissue is seen connecting the infundibular process to the brain just in front of the mammillary bodies. The upper half of this band is filled with nerve cells, whereas the lower half includes only a few. The line of demarcation between these areas is fairly sharp and the lower half only will be considered as the posterior limb of the stalk. As Rioch *et al.* point out (p. 25), the general limits between the infundibular process and infundibular stalk seem clear enough.

A careful study of the neurohypophysis of the dog, following Nissl staining, haematoxylin and eosin and the Hortega silver carbonate methods, shows conclusively that the bulk of the infundibular process is made up of pituicytes. Following the first two staining methods the pituicyte nuclei are well stained, but their cytoplasm and processes remain unstained. Only after silver carbonate impregnation are the processes of these cells demonstrated (Bucy, 1930; Shanklin, 1940). The nuclei of the pituicytes are small and nucleoli are lacking. The nerve cells are much larger and have abundant cytoplasm filled with Nissl bodies. The nucleoli

of the nerve cells are very large and darkly stained.

The total number of nerve cells in the neurohypophysis is extremely variable; the same is true regarding nerve cells in its different parts. In order to avoid duplication only nerve cells were counted in which nucleoli were seen. Counts are reported on the hypophyses of three dogs (see Table 1).

Table 1

	Total no. sections	Total no. cells	Average	Range
	Hypophysis D			
Anterior limb	164	71	0·4	0– 5
Posterior limb	164	106	0·6	0– 6
Junction	124	55	0·4	0– 7
Infundibular process	240	10	0·1	0– 2
	Hypophysis F			
Anterior limb	116	116	1·0	0– 8
Posterior limb	116	37	0·3	0– 3
Junction	101	161	1·6	0– 6
Infundibular process	277	157	0·6	0–10
	Hypophysis G			
Anterior limb	88	234	2·7	0–15
Posterior limb	88	413	4·7	0–12
Junction	69	315	4·6	0–11
Infundibular process	226	149	0·7	0– 9

The nerve cells found in the anterior and posterior wall of the stalk are scattered at random, but those in the infundibular process are found most frequently in the part proximal to the junction. However, nerve cells were observed in all regions of the process.

Nerve cells in the stalk, at the junction, and in the infundibular process proximal to the junction were usually located parallel to the descending nerve fibres. There are some exceptions; for example, a very large cell with a long process was located in the lower part of the stalk with its long axis at right angles to the fibres.

The diversity of shapes of these nerve cells is considerable. A few of them are round, others multangular and some tend to be fusiform. Their nuclei and nucleoli are uniformly large. Although the nucleus is usually in the centre of the cell, a few nuclei are eccentrically placed. For the most part the Nissl bodies are concentrically arranged around the nucleus, but in several specimens these bodies are lacking in a considerable part of the cell.

The sizes of these nerve cells cover a wide range. For example, one cell was found to have a diameter of $20 \times 25\,\mu$, another $15 \times 37\,\mu$, and another $14 \times 35\,\mu$. The nerve cells in the neurohypophysis are, on the average, fully as large as the majority of those in the hypothalamus. A series of cells measured at random in the hypothalamus of dog F showed some cells as small as 10×11 and $10 \times 13\,\mu$, while the large ones were of such magnitude as 15×32 and $20 \times 40\,\mu$.

After this paper had been completed the writer saw the brief description of nerve cells in the neurohypophysis of the porpoise by Geiling, Vos & Oldham (1940). Their description (p. 310) is as follows: 'In addition to the pituicytes, another cell type is seen in the proximal portion of the neural lobe and in the neural stalk of both the bottle-nosed and the speckled porpoise. This cell has a large vesicular nucleus and an irregularly dispersed cytoplasm, which stains a fairly deep blue with hematoxylin-eosin-azure II (fig. 7). These cells bear a striking resemblance to the nerve cells in the hypothalamus.'

SUMMARY

Typical nerve cells have been identified in all parts of the neurohypophysis of the dog.

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THE BLOOD SUPPLY OF NERVES

II. THE EFFECTS OF EXCLUSION OF ITS REGIONAL SOURCES OF SUPPLY ON THE SCIATIC NERVE OF THE RABBIT

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The probability that a constant and adequate supply of blood is not the least of the essential functional requirements of a nerve (see Adams, 1942) immediately leads us to inquire: Is a nerve, then, more dependent on its regional or on its longitudinal sources of supply? In the literature, apart from Koch (1926) who was reluctant to consider the regional sources as indispensable, I can find only one other investigator who has considered this problem: Okada (1905) has affirmed that occlusion (by ligation) of a single nutrient artery results in degeneration of the nerve which it supplies. This is a significant thesis and is entitled to our most serious consideration for, if degeneration may thus supervene in a nerve whose axis cylinders retain anatomical continuity with their cell bodies, it would be necessary in many cases to dissociate *ischaemic* from Wallerian degeneration; and again, if such degeneration be substantiated, we might reasonably anticipate comparable effects whenever a length of nerve is mobilized without regard for its nutrient vessels. Moreover, in the repair of certain lesions of peripheral nerves where separation of a nerve from some of its regional sources of supply must frequently occur, there is a possibility of serious impairment of the normal process of regeneration in both the central and peripheral stumps. From every angle, whether it be the normal functioning of intact peripheral nerves or one of the many related surgical and medical problems, the subject is clearly one of considerable interest.

Let us then examine briefly the relevant evidence. Okada studied the effects, in the rabbit's sciatic nerve, of ligation of the inferior gluteal artery above the origin of its nutrient branch, the arteria 'comitans' nervi ischiadici, which—although admitting that it is not the only vessel supplying the nerve—he regarded as the 'largest, the most significant and the most constant of all the arteries of this nerve'. Those other vessels, however, which contribute to the intraneural plexus of the sciatic nerve are by no means as insignificant as he would suggest; indeed, they may be even more important than the inferior gluteal (Koch). In excluding such vessels, therefore, merely because they do not pass directly to the sciatic nerve, Okada has disregarded two established facts: (1) that the division between the sciatic nerve and its branches is purely descriptive since it is well known that the apparent branching of the nerve is reflected in its internal organization long before the individual branches separate; and (2) that the intrinsic vessels of the main sciatic nerve are quite continuous, anatomically and physiologically, with those of its 'terminal' branches. So, in view of this vascular and nervous continuity between the sciatic nerve and its branches, the adoption of a rigid line of demarcation between them is, to say the least, liable to be very misleading, for vessels which undoubtedly contribute to the total vascularity of the nerve may thus be ignored simply because they reach it, not directly, but by way of its branches.

Careful consideration of the operative technique is essential if we wish to assess accurately the results of such an experiment. The inferior gluteal artery is not difficult to identify; on the other hand, it is not easy to ligate it above its upper nutrient ramus (Okada disregards the fact that there are generally two such branches) without damaging the nerve, because the artery, lying under cover not only of the sciatic nerve but also of the inferior gluteal vein and the nerve to femoro-coccygeus muscle, is usually relatively inaccessible at this level. Okada says that the nerve 'was detached from its bed in order to dispose of any other sources of supply difficult to identify' although 'strictly avoiding any injury to the nerve'. Whether this was a routine procedure is not quite clear, but where it is specified we cannot disregard it for possibility of injury to the nerve can only be certainly excluded by avoiding it altogether. Okada ligated the inferior gluteal artery in fourteen cases, in four of which he records quite definitely that he separated the nerve from surrounding structures; the inadequate description of his operative procedure, however, does not help to dispel the suspicion that in his other animals also some manipulation of the nerve might possibly have taken place.

Although there are many other points which might be criticized, Okada has undoubtedly produced evidence of degeneration in the majority of his cases. His animals were left after operation for periods varying from 4 to 111 days. The animal killed after 4 days showed very few degenerated fibres, but by the 5th and 6th days degeneration was more evident; subsequently degenerated fibres predominated and in many cases the nerve was completely degenerated. He studied the changes in the medullary sheaths by teasing after staining with osmic acid and by the Marchi method, while those

in the axis cylinders were investigated by the van Gieson technique, a method which certainly does not favour his claim to have observed regeneration of fibres in some of his longer experiments.

The clinical signs were merely summarized and were not correlated with the histological changes; they are frequently unconvincing. Thus, in spite of the complete degeneration of the sciatic nerve which was often obtained, total paralysis of the leg was never observed; and in view of the results obtained by Gutman & Guttman (Gutman, Guttmann, Medawar & Young, 1942) in lesions of the peroneal nerve alone, the extent of analgesia seems rather restricted for the extensive disturbance of the sciatic nerve which Okada claimed to have produced.

His results, therefore, although certainly dramatic, are not by themselves entirely convincing, and they require independent confirmation. For that reason I have repeated the original experiment and carried out a further elaboration, viz. a study of the effects of occlusion of *several* sources of supply of the sciatic nerve. The present paper records the results of these experiments.

MATERIAL AND METHODS

For convenience the rabbit was again used. A careful investigation was first made of the vascular connexions, in the thigh, of the sciatic nerve and its branches (Text-fig. 1). Apart from minor variations, three principal sources of blood supply were identifiable:

1. *Proximal.* Soon after it enters the thigh from the pelvis the sciatic nerve receives a slender branch or branches from the inferior gluteal artery; generally they are two in number and pass laterally from the artery to the nerve. Since the artery progressively diverges from the nerve, the lower of the two nutrient arteries is the longer and (in accordance with Bartholdy's dictum, 1897) is also the larger—it is this vessel presumably which has been called the arteria 'comitans' nervi ischiadici. The two nutrient arteries approach the medial side of the nerve a short distance below the pyriformis muscle and divide on its surface into ascending and descending rami (usually multiple) of which the latter are the larger. The ascending rami, of the upper nutrient artery especially, anastomose with small vessels which accompany the sciatic nerve from the pelvis. Thus the continuity of the intraneural plexus is maintained. The principal descending ramus of the lower nutrient artery passes down not with the sciatic nerve but with its branch to the hamstring group of muscles, although during its course with the latter it provides branches which run transversely outwards to rejoin the main nerve, again dividing there into ascending and descending rami.

2. *Intermediate.* A small artery, a perforating branch of the profunda femoris artery, reaches the back of the thigh in company with the nerve to the adductor magnus muscle. It generally reaches the sciatic nerve at its medial side, dividing as usual into ascending and descending rami of which the former anastomoses with the descending rami of the proximal source. Occasionally (as in Text-fig. 1) this nutrient vessel joins a very slender vessel which appears through the adductor longus muscle, and finally reaches the nerve at its lateral side.

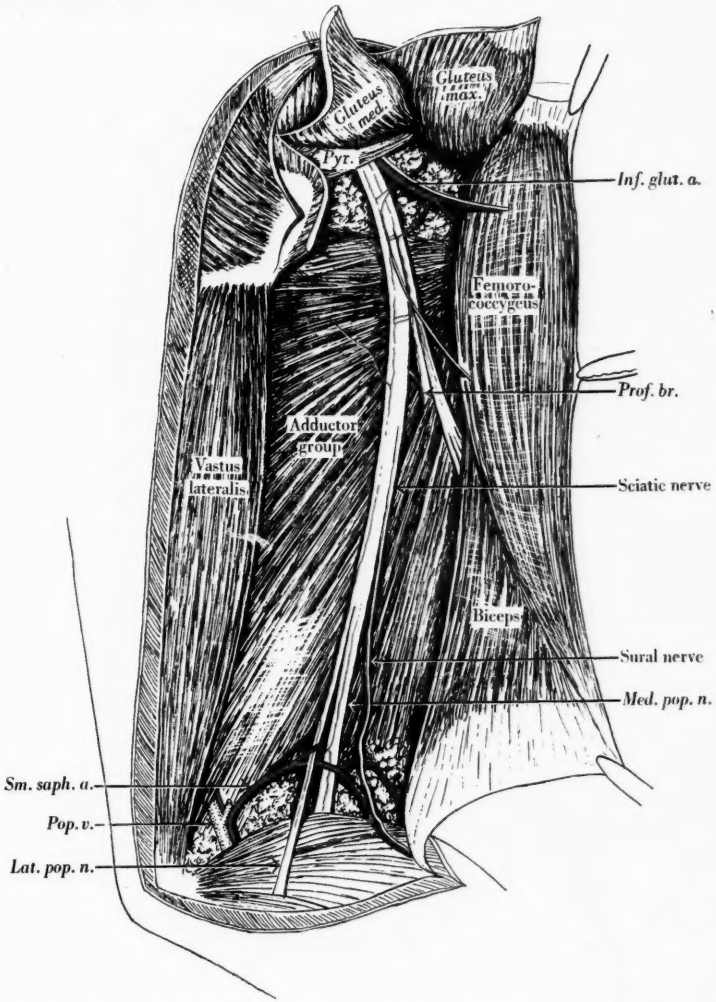
3. *Distal.* The distal supply is derived entirely, by slender ascending branches, from the small saphenous artery. These nutrient arteries are invariably three in number and accompany the medial and lateral popliteal and sural nerves (Pl. 1, fig. 1). They ascend for some distance on the surface of these nerves before piercing the epineurium and disappearing into the interfascicular connective tissue; they obviously contribute to the intraneural plexus of the sciatic nerve and in this way communicate with the intermediate and proximal sources of supply.

The veins draining the sciatic nerve always accompany the arteries; they are more obvious than the latter owing to their larger calibre, and where this is increased, as under nembutal anaesthesia, they tend to dominate the vascular picture.

Examination of the nerve after injection with India ink and clearing (Pl. 1, fig. 1) confirms the fact that these nutrient arteries help to form a continuous longitudinally disposed plexus of vessels which lies in the interfascicular connective tissue and is continued upwards into the pelvis. From this primary plexus (Pl. 1, fig. 3) are derived the capillary plexuses of the nerve, which are situated in the endoneural connective tissue. Although every nerve fibre is not in relation with capillaries, nevertheless the plexus is not as sparse as is generally believed. These results agree with those recorded in a variety of mammals by Valentin (1920), who was also able to show that nerve fibres occasionally lie in direct relationship with the capillary plexus.

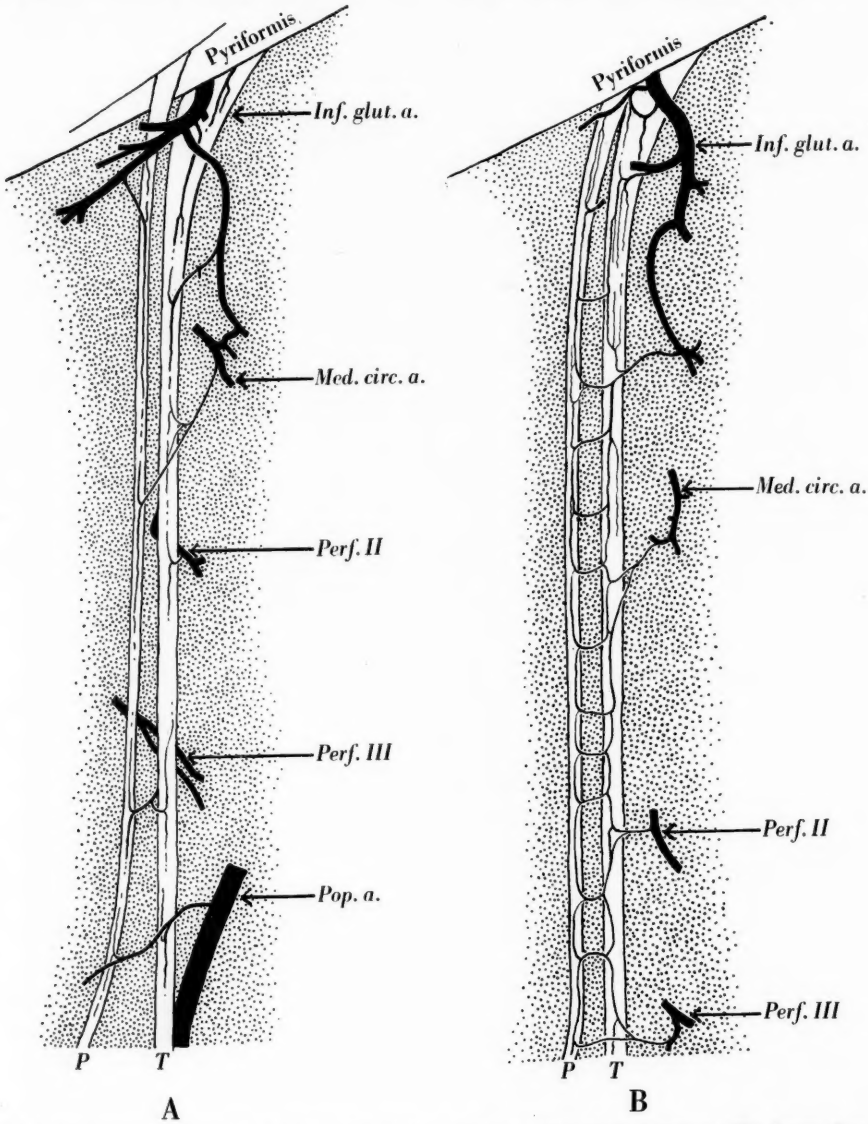
It must not be assumed from the above that such arterial arrangements obtain universally in the mammals. The vascular connexions of the human sciatic nerve, for example, have been studied by numerous investigators (Tonkow, 1898; Bartholdy, 1897; Hofmann, 1903; Chaumet, Heyman & Mouchet, 1921; Bleicher & Mathieu, 1934; Buitink, 1934; Picco, 1936), whose researches show that some differences in detail exist between the conditions in the rabbit and those in man. The absence normally in man of a small saphenous artery naturally deprives the branches of the sciatic nerve (and therefore the sciatic nerve itself) of those conspicuous vessels which we have seen in the rabbit ascend from this artery along the medial and lateral popliteal and sural nerves. According to the excellent description given by Hofmann, this defect is compensated for by an increase in the number of

nutrient arteries which the two divisions of the sciatic nerve receive in the thigh—for the perforating arteries as well as the medial femoral circumflex and inferior gluteal all appear to send nutrient vessels to the sciatic nerve. Thus the medial popliteal nerve may receive eight successive nutrient arteries and the lateral popliteal four (Text-fig. 2A). Frequently, however, a further variation is encountered in the human sciatic nerve, in which the lateral popliteal division derives its blood almost entirely by way of the longitudinal vascular pathway within the medial popliteal nerve, through numerous arcuate vessels passing from the one to the other (Text-fig. 2B). Hofmann attributes to this reduced blood supply the well-known greater vulnerability of the lateral popliteal division of the



Text-fig. 1. Left thigh of the rabbit showing the sciatic nerve as exposed at operation in its normal relations. Nutrient arteries are figured both approaching the nerve and upon its surface; no attempt has been made to show their ultimate ramifications. Nutrient veins have been removed. The following abbreviations are used for this and the next figures: *Inf. glut. a.* = inferior gluteal artery; *Pop. a.* = popliteal artery; *Pop. v.* = popliteal vessels; *Med. circ. a.* = medial femoral circumflex artery; *Sm. saph. a.* = small saphenous artery; *Prof. br.* = nutrient branch of profunda femoris artery; *Perf. II, III* = perforating branches of profunda femoris artery; *Med.* (*Lat.*) *pop. n.* = medial (lateral) popliteal nerve.

sciatic nerve, particularly where it has been subjected to stretching; although an important factor may also be the more slender longitudinal pathway in this nerve, since he considered that this would be much more liable to occlusion in elongation of the nerve than the more robust anastomotic plexus of the medial popliteal division. His conception of the vascular independence and individuality of the two main divisions of the sciatic nerve is a most interesting one, and it may have far-reaching implications.



Text-fig. 2. The vascular connexions of the human sciatic nerve in the thigh (after Hofmann). The figures show the two principal types of vascularization: A, in which the medial (*T*) and lateral (*P*) popliteal nerves receive their nutrient vessels independently; and B, in which the vascular supply of the lateral popliteal nerve is derived from that of the medial. Other abbreviations as for Text-fig. 1.

In my experiments twenty rabbits were investi-

gated. Anaesthesia was induced by intravenous nembutal prolonged when necessary by the administration of ether. The approach to the sciatic nerve was by the conventional (lateral) route: after incision of the skin the femoro-coccygeus and biceps muscles were reflected from their attachments anteriorly, along a curved line commencing at the coccyx, extending outwards to the great trochanter, thence downwards on the outer side of the thigh, past the patella to the outer aspect of the leg. In this way only the aponeurotic attachments of these muscles are severed. The muscles were then retracted backwards after ligation of the branches of the small saphenous vessels which supply them. The gluteus maximus was then detached from its insertion into the third trochanter, and its posterior moiety separated and reflected upwards. Reflexion then of the gluteus medius muscle from the great trochanter resulted in an almost complete exposure of the sciatic nerve in the thigh (Text-fig. 1). Ligation of the inferior gluteal artery was however considerably facilitated by the reflexion of the pyriformis muscle also.

The nerve having been thus exposed, one of the following procedures was then carried out:

(1) *Ligation of the inferior gluteal artery alone.* Where it enters the thigh this artery is intimately related to the sciatic nerve and the nerve to femoro-coccygeus; it is usually under cover of both these nerves and the inferior gluteal vein. Its ligation above its nutrient branches demanded considerable care; in the first place it was difficult at this level to separate it from the vein so that quite often the two had to be ligated together (a difficulty apparently encountered by Okada); secondly, ligation of the artery at this level needed constant vigilance to avoid any interference with the sciatic nerve which partly overlaps it. In certain cases the artery was ligated below as well as above its nutrient vessels to obviate the establishment of a collateral circulation from below.

(2) *Ligation of all sources of supply in the thigh.* The inferior gluteal artery was ligated as described above. The profunda vessels (both arteries and veins because it was impossible to separate them) were then tied off. Finally the distal sources were eliminated by ligation of the small saphenous artery close to its origin from the femoral; in a few cases this vessel was again ligated beyond its nutrient branches to prevent a possible re-establishment of circulation from below.

After operation the animals were left for periods varying from 6 days to 1 month, and were then killed. The nerves were examined histologically by the Marchi method, and by Bodian's technique both alone and in conjunction with Masson's stain. In every case the whole length of the nerve and a considerable part of the medial and lateral popliteal nerves were studied.

OBSERVATIONS

1. *Ligation of the inferior gluteal artery*

In the eight animals in which the inferior gluteal artery was ligated great care was taken to prevent any injury to the nerve; where necessary the artery and the vein were ligated together. In one animal the nerve was purposely freed very carefully from its surroundings for some distance in the region of the inferior gluteal artery. In three cases, in addition to the proximal ligation, the artery was also ligated distally. After operation the animals were left for periods varying from 6 days to 1 month, during which they were frequently examined for any signs of neurological disturbance which might have resulted from the operation.

Results. None of the animals showed any disturbance of movement or of sensation, apart from a slight flexion of the thigh which was frequently observed in the earlier stages and was regarded as a post-operative effect. Reflex movements such as spreading of the toes were unaffected, but it should be pointed out that, according to Young and his collaborators (see Gutman *et al.* 1942), the toe-spreading reflex indicates only the integrity of the lateral popliteal division of the sciatic nerve, a view which has received recent support from the work of Altschul & Turner (1942). Histological examination of the sciatic nerve and of its branches disclosed generally no degeneration whatever or, at the most, degeneration of relatively few solitary fibres (Pl. 2, fig. 4). No connective tissue proliferation or other obvious histological change was observed.

2. *Ligation of all sources of supply*

In twelve animals the inferior gluteal artery, the branch of the profunda femoris artery and the small saphenous artery were all ligated, the first and last of these proximal to the origins of their first nutrient rami. Except in three cases (in which the nerve was also carefully freed from surrounding structures) there was no interference with the nerve. As previously, the animals were left after operation for periods of 6 days to 1 month and they were similarly examined during this period.

Results. In two animals only was any gross abnormality detected. Of the other ten, seven showed no clinical or histological signs whatever of degeneration of the sciatic nerve or of its branches; in the other three, however, none of which had presented any clinical signs of involvement of the nerve, the latter showed a variable but small number of degenerated fibres. In the one of this group (B.C., 14 days) which showed the greatest number of degenerated fibres (Pl. 2, figs. 5, 6), the nerve had, in addition to the ligation, been freed from its surroundings, and the superficial disposition of the degenerated fibres indicates that this interference was possibly responsible.

In the two cases in which clinical signs were clearly observed the ligation had also been accompanied by mobilization of the nerve. Both animals (R. 5, 11 days; B.D., 6 days) showed marked flaccid paralysis of the muscles of the foot and leg, loss of the toe-spread reflex, and analgesia of the foot and lower leg. Histological examination showed, in both cases, that the sciatic nerve and its branches had undergone extensive degeneration (Pl. 2, fig. 7; Pl. 3, figs. 8, 9, 10). If these two cases are compared with the previous one (B.C.) in which mobilization of the nerve was also carried out but in which the degenerative changes were comparatively slight, there seems to be some justification for attributing the much more extensive degeneration here to the effects of the 'devascularization'. Mobilization of a nerve if it is carefully executed, and the nutrient vessels preserved, does not, in my experience, involve many fibres, and has certainly never resulted in the massive changes obtained in these two animals. This view, moreover, is supported by the interesting experiment carried out by Torraca (1920), who, after mobilizing the sciatic nerve in the dog, stripped it of its epineural sheath and surrounded it with a rubber covering. The fact that only a transitory paralysis resulted, and that full power of movement soon returned, indicates clearly that quite extensive interference with the nerve can be carried out without ill effects ensuing. It is interesting to note that Torraca considered the intrinsic (i.e. longitudinal) circulation of an 'isolated' nerve is sufficient to preclude any prospect of serious disturbance to the nerve in such an experiment. In the present experiment the fact remains, however, that degeneration occurred only in the minority of the animals investigated. Is it possible that this may be attributable to variations in the vascularity of the nerve, and that in these two cases the nerve was dependent to a greater degree upon its regional sources of supply? Such a possibility at least demands further investigation.

In one of these two animals (B.D.) the nerve of the opposite leg (right) was sectioned at the time of operation. Examination of the two nerves showed that the effects on the two sides were quite comparable in regard to both the extent and the severity of the degeneration; the clinical signs were also equivalent. A careful study of the 'devascularized' nerve (Pl. 3, fig. 10) and its comparison with the sectioned nerve (Pl. 3, fig. 11) show that the changes in it are just those which might be expected of a sectioned nerve undergoing typical Wallerian degeneration after a corresponding interval of time (6 days), i.e. medullary changes are well advanced, fragmentation of the axis cylinders is generalized, and the proliferation of Schwann cells has just commenced. If then these results are attributable to the 'devascularization', the effects of this procedure must correspond closely to those of typical Wallerian degeneration. Unfortunately it was not possible to determine whether the transition between the normal part of the nerve above, and the degenerated part below, was a sudden or a gradual one.

DISCUSSION

As far as ligation of the inferior gluteal artery alone is concerned it is clear that there is a striking discordance between my results and those of Okada. Whereas he claimed in every case to have produced histological changes in the sciatic nerve, in not one of my series has any significant change resulted from ligation of the inferior gluteal artery alone. My results therefore do not support his contention that occlusion (by ligation) of this source of blood supply materially affects the nerve. Furthermore, in the majority of the animals in which ligation of all sources of supply in the thigh was carried out, the nerve showed either no change at all or none of any significance. In two cases, however, massive degeneration of the nerve did occur; in both of these there had been some additional manipulation although scrupulous care was taken to avoid actual damage to the nervous elements themselves. While I hesitate to attribute solely to trauma the extensive lesions which were observed, nevertheless it cannot be disregarded as a possible causative factor. The possibility of variations in the vascular supply of the nerve must, however, also be considered, for a nerve which is unduly dependent upon its contributory sources of blood supply must surely react more readily to their obliteration than one in which the intrinsic longitudinal pathway can satisfy its normal requirements.

These results therefore justify the assumption that severe disturbance is unlikely to proceed from mobilization of a length of peripheral nerve if this should involve the interruption of only one nutrient vessel; but in the case of more extensive separation of a nerve the effects are rather more uncertain and the results may then depend much more upon variations in blood supply both in the same nerve and as between different nerves. This important aspect of the problem is being pursued.

In discussing this problem, however, we must bear in mind two important considerations:

(1) Manifest degeneration in a nerve signifies a gross disturbance both of structure and of function; it is a change, moreover, which is irreversible. But the absence of degeneration does not necessarily signify that no change at all has occurred within the nerve since there always remains the possibility of *physiological* or reversible changes unassociated with any obvious disorder of the nerve fibres. Evidence from other sources (Lewis, Pickering & Rothschild, 1931) suggests that such physiological changes may occur as a result of ischaemia of nerve and that they may even give rise to objective signs comparable in many ways (but not in their progression) with those resulting from traumatic inter-

ruption of a nerve; in such cases the alterations in the functional capacity of the nerve fibres must apparently fall far short of those necessary to produce actual degeneration since the function both of the nerve and of the parts dependent upon it may be rapidly restored. Nevertheless, it would be interesting to know whether any histological change does occur in the nerve as a result of such temporary ischaemia. In connexion with this the possibility that persistent objective signs may occur in the absence of obvious histological signs of degeneration is mentioned by Aring, Bean, Roseman & Spies (1941), who state that in pellagrins 'mild clinical signs may occur in the face of an apparently normal myelin-sheath content of the nerve', although their evidence for this is not entirely satisfactory.

Relevant also to this discussion is the excruciating pain which is a characteristic symptom of thromboangiitis obliterans and which has been attributed to ischaemia of the nerves involved consequent on thrombosis of their vasa nervorum. Meleney & Miller (1925) found that in every case which showed occlusion of the vasa nervorum pain was present, but there were no demonstrable changes in the nerve fibres of the affected nerves. But other workers who likewise attribute the pain to ischaemia of the affected nerve trunks have reported otherwise; Barker (1938), for example, found that occlusion of the vasa nervorum (both arteries and veins) was associated with changes in the nerves which he calls 'Wallerian degeneration'. The lesions, however, do not appear to me to be at all characteristic of typical Wallerian degeneration since (after 2 months or more) 'variations were noted from patchy fragmentation of the myelin to complete loss of myelin and destruction of the entire architecture of the nerve with replacement by fibrous tissue'; while 'the axis cylinders either appeared normal or could not be identified at all' and the presence of Büngner's bands is not mentioned (nor do they appear to be evident in certain of his figures).

There can be little doubt that the difficulty of explaining the symptoms in this and many other allied clinical conditions is due partly to our inadequate knowledge of the physiological results of ischaemia of nerve, and partly to the difficulty of correlating the physiological facts at present at our disposal with what we know of the anatomical characteristics of the blood supply of nerves. The recent researches of Rosenblueth and his collaborators (Rosenblueth & del Poso, 1942) on circulated mammalian nerve suggest that further extensive and careful investigation along such lines would not be unprofitable.

(2) Ligation of such sources as the inferior gluteal or small saphenous arteries does not necessarily deprive a nerve of a continuous supply of blood. The longitudinal vascular pathway which every nerve possesses still retains its continuity through the affected zone, and still presumably remains patent. Although ligation of a source of supply may produce a temporary local diminution of blood supply it is difficult to conceive how the effect could be other than a transient one, especially in view of the facility with which the longitudinal pathway is capable of enlarging and thus compensating for the local loss. So, unless the longitudinal anastomosis should prove inadequate to accommodate itself to the changed conditions (and this may conceivably be brought about by stretching of the nerve as Hofmann suggests), there is little reason to expect extensive degeneration of the constituent nerve fibres; but the frequency with which the longitudinal pathway may provide a collateral circulation not only to the nerve itself but even to a whole limb (Holl, 1880; Zuckerkandl, 1885; Tonkow, 1898; and others) clearly indicates its potentialities in this respect. Nevertheless, although ligation of a regional source might hardly be expected to have any pronounced or lasting effect upon the nutrition of the nerve it supplies, the conditions must surely be quite different if the occlusion of a nutrient artery is not localized (as it is in the case of a ligature) but involves all its intraneural ramifications (as for example in thrombosis). For then not only would the regional sources be abolished but at the same time the continuity of the longitudinal vascular pathway would be interrupted and the flow of blood through the affected region arrested. An infarction of the nerve thus produced, and the consequent prolonged local ischaemia, might well result in a severe destruction of nervous tissue. Indeed, this is implied in Gammel's explanation (1927, 1928) of the paralyses observed in the condition of Embolia cutis medicamentosa, although in his cases it is quite possibly not the whole story.

While therefore my results suggest very strongly that *remote* interference with the regional sources of blood supply of a nerve has generally no pronounced effect upon the nerve, they do not exclude the possibility of more serious involvement if the occlusion of a nutrient vessel should embrace all its intraneural ramifications, and in this way interrupt the longitudinal vascular pathway in addition. This aspect of the problem is now being investigated.

SUMMARY

1. The blood supply of the sciatic nerve in the thigh of the rabbit is described, and contrasted with that of man.

2. Contrary to Okada's contention that degeneration invariably results from ligation of the inferior gluteal artery, in eight rabbits in which this operation was repeated no degeneration or other obvious histological change occurred.

3. An extension of the experiment to include the other sources of supply of the sciatic nerve in the thigh was carried out in twelve animals. In ten of these there was either no degeneration at all, or if

present it was extremely limited and insignificant. In the other two cases, in both of which the nerve had also been mobilized, extensive degeneration with concomitant clinical signs resulted. It is uncertain however whether these two results are to be attributed to the slight manipulation of the nerve, or to some variation in its intrinsic vascular pattern in these cases.

4. The significance of these results is discussed.

I am particularly grateful to Prof. A. Durward of this University for his stimulating encouragement and continued interest in the progress of this work, and to Prof. H. A. Harris of the University of Cambridge for the kind hospitality offered to me by his Department in the initial stages. I am indebted also to Mr A. W. Dent, of this Department, for his generous help at all stages of the work and particularly for the preparation of the histological material; and to Mr J. Manby, of the University of Leeds, for his care in preparing the photomicrographs.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Injected and cleared specimen of the lower part of sciatic nerve showing the vascular anastomoses between the nutrient branch (*P.A.*) of the profunda femoris artery and those of the small saphenous artery (marked by arrows), which are running with the medial (*T*) and lateral (*P*) popliteal and sural (*S*) nerves. ×5.

Fig. 2. Transverse section of the lateral popliteal nerve showing the nutrient branch (*Nut.A.*) of the small saphenous artery which ascends along it to the sciatic nerve. Bodian-Masson. ×75.

Fig. 3. Injected and cleared specimen of part of the sciatic nerve showing the interfascicular (precapillary) plexus. Larger epineural vessels are also evident. ×50.

PLATE 2

Fig. 4. Rabbit A.F. (6 days). Ligation of the inferior gluteal artery. Longitudinal section of the medial popliteal division of the sciatic nerve showing solitary degenerated fibres. Not more than 100 degenerated fibres were counted in the entire nerve. Marchi. ×150.

Fig. 5. Rabbit B.C. (14 days). Ligation of all sources. Transverse section of the medial popliteal division of the sciatic nerve in its lower third. The superficial location of the degenerated fibres is evident. A slight haemorrhage is noticeable in the upper part of the section. Marchi. ×60.

Fig. 6. The same. Longitudinal section at a higher level showing the degenerated fibres (*D*) lying superficial to the underlying normal fibres (*N*). Bodian-Masson. ×150.

Fig. 7. Rabbit R. 5 (11 days). Ligation of all sources. Representative longitudinal section of the sciatic nerve showing advanced degeneration of medullary sheaths, which characterizes the entire nerve. Marchi. ×150.

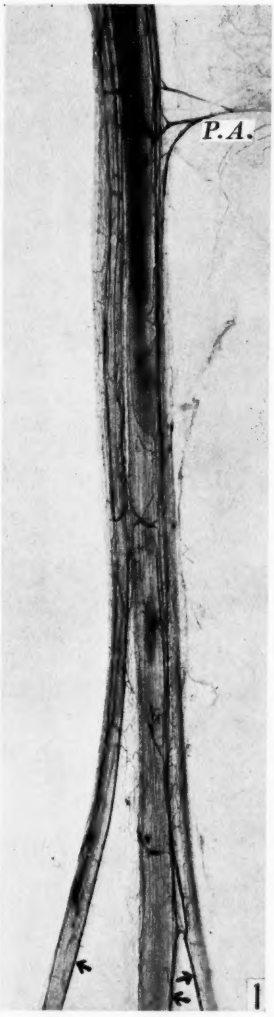
PLATE 3

Fig. 8. Rabbit B.D. (6 days). Left sciatic, ligation of all sources. A representative longitudinal section of the medial popliteal nerve showing extensive destruction of medullary sheaths. Marchi. ×150.

Fig. 9. Rabbit B.D. Left sciatic. Representative longitudinal section of the lateral popliteal nerve also showing extensive medullary changes, but not at such an advanced stage as those seen in the medial popliteal. Marchi. ×150.

Fig. 10. Rabbit B.D. Left sciatic. Representative longitudinal section showing the marked fragmentation of the axis cylinders and formation of 'digestive chambers'. Proliferation of Schwann cells just commencing. Bodian-Masson. ×150.

Fig. 11. Rabbit B.D. (6 days). Right sciatic, sectioned. Longitudinal section of peripheral cut end, showing fragmentation of axis cylinders and commencing proliferation of Schwann cells. For comparison with fig. 10. Bodian-Masson. ×150.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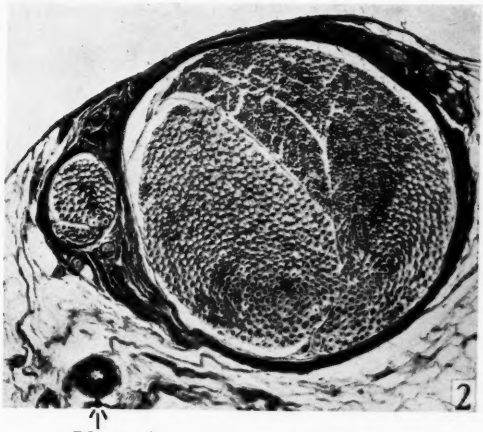
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THE PROBLEM OF NEURONAL REGENERATION IN THE CENTRAL NERVOUS SYSTEM

II. THE INSERTION OF PERIPHERAL NERVE STUMPS INTO THE BRAIN

By W. E. LE GROS CLARK, *Department of Anatomy, University of Oxford*

In a previous communication (Clark, 1942) the results of implanting in the brain of portions of predegenerated sciatic nerve were recorded. In some experiments regenerating axons were found to have penetrated into the implant. They were very few, however, and it was concluded that, if the intrinsic fibres of the rabbit's brain have any regenerative capacity at all, it is relatively feeble in comparison with that of peripheral nerve fibres. On the other hand, the experiments provided a strong presumption that the regenerating fibres were derived, not from the new growth of intrinsic fibres of the brain, but from other sources such as vascular nerve fibres and peripheral fibres in the meninges or scalp tissues. In other words, no conclusive evidence was obtained for the view of neurohistologists such as Cajal (1928), Tello (1911), and Ortin & Arcaute (1913), that the tissues of a predegenerated peripheral nerve induce the active regeneration of nerve fibres in the central nervous system. In criticism of the experiments it may be justly argued that, since the portions of predegenerated nerve were taken from other animals (homografts), and since the vitality of their cellular elements would be seriously affected by the absence of all blood supply until they had become secondarily vascularized, the influence which they may otherwise exert on nerve regeneration would be at least greatly diminished. To overcome such difficulties a further series of experiments has been completed. In this series either the proximal or the distal stump of a cut peripheral nerve was directly inserted into the brain of the same animal. Thus the advantages of an autograft were combined with the maintenance of a vascular supply (even though the latter was probably diminished to some degree by the preliminary mobilization of the nerve). Further, the insertion into the brain of the proximal stump of a peripheral nerve permitted observations on the behaviour of axons growing from the cut end of the nerve when they come into relation with the tissues of the central nervous system.

METHODS

The experiments were carried out on rabbits under intravenous nembutal anaesthesia, using strict aseptic precautions. In the insertion of the proximal stump of a peripheral nerve, the facial nerve was employed in five animals. An incision was first made along the course of the nerve over the surface of the masseter muscle. The nerve was lifted up and cut as far forwards as possible. Another incision was then made over the temporal region. The temporal muscle was separated from its bony origin with a raspatory, and a small trephine hole was drilled in the underlying skull. The proximal stump of the facial nerve was then drawn under the skin up to the position of the trephine hole, and with a pair of fine forceps it was pushed through the dura mater and into the substance of the brain. The inserted nerve was sealed in position by a few drops of concentrated plasma (for the preparation of which I am grateful to my colleague Dr Medawar). The incisions were then closed. In some cases it was found that the parotid duct, which is closely adherent to the facial nerve, had also been inserted into the brain. As will be seen, the proliferation of its lining epithelium in the brain tissues was not without interest. In three animals the facial nerve was crushed at the most proximal part of its facial course before insertion. For the insertion of the distal stump of a peripheral nerve, the occipital nerve was used in four animals. This nerve, which is small but of convenient size, is the posterior primary ramus of the second cervical nerve. It extends up the back of the neck to the occipital region of the head and finally passes on to the medial margin of the ear. The nerve was exposed by a longitudinal incision to one side of the midline. It was cut at the point where it emerges from the nuchal musculature, and the proximal end of the distal stump was inserted into the occipital region of the cerebral hemisphere through a small trephine hole in the skull. Since the nerve was long enough to be inserted into position without tension, sealing with plasma was not necessary. The incision was closed in the usual way. In one animal, the inserted nerve was exposed at a second operation one week before death and sectioned close to the skull to ensure degeneration of any regenerated axons which might have grown down in a recurrent direction.

The animals were kept alive for periods varying from 1 to 6 weeks, and were killed by decapitation. In each experiment a block containing the site of insertion was removed from the brain and serial sections were cut at a thickness of 10μ. In most cases the block was treated by a pyridine-silver technique; material of two of the experiments was stained by Bodian's protargol method.

EXPERIMENTAL RESULTS

R 230. Proximal stump of facial nerve inserted into brain, and animal killed 3 weeks later. Pyridine-silver sections show that the nerve extends through the cortex of the parietal lobe, traverses the medial margin of the lateral ventricle, and enters the underlying subicular cortex. The main cut end of the stump is imbedded in the white matter adjacent to the hippocampus. Although the nerve had not been crushed at the operation, the axons in the implanted portion had evidently undergone complete degeneration and are replaced by regenerated axons, which are present in great abundance. In most of the fasciculi the growth of the regenerated axons has been obstructed by fibrous tissue at their cut ends, and here the fibres form a tangled skein from which they extend their growth by running spirally round the inner aspect of the perineural sheath (Pl. 1, fig. 5). At the free end of the main stump of the implanted nerve there has been a considerable local reaction in the adjacent tissues of the brain; a circumscribed zone of granulation tissue, containing many macrophages and an infiltration of closely packed small round cells, extends down for a short distance into the subicular cortex. Penetrating into this zone of inflammatory tissue are numerous strands of Schwann cells which have grown out from the cut end of one of the fasciculi of the facial nerve; they form in some cases relatively thick bundles of closely packed cells and in others fine chains of single Schwann cells, and they are disposed in a loose plexiform arrangement. Extending out along many of these strands are fasciculi of actively growing axons which can be seen streaming out from the cut end of the implanted nerve (Pl. 1, fig. 1). They run for a distance of about 1 mm. into the granulation tissue and finally terminate in fine beaded fibres which cling closely to the fine terminal strands of Schwann cells (Pl. 1, fig. 2). In some cases the free ends of these regenerated fibres appear to run into the granulation tissue without accompanying Schwann cells, but the histological picture as a whole suggests very forcibly that the proliferating strands of Schwann cells (many of which have no axons in relation to them) serve as guides for the subsequent outgrowths of regenerating nerve fibres.

The cellular infiltration of the subcortical white matter in the neighbourhood of the stump of the implanted nerve has led to the local destruction and disappearance in this situation of the fibre plexuses of the brain. However, scattered fibres are found still persisting in the marginal parts of the infiltrated zone, particularly along the course of blood vessels where, presumably, their survival is facilitated. In some sections the extensions of such fibres from the adjacent intact white matter along blood vessels into the zone of cellular infiltration gives the appearance of an actual outgrowth of new fibres from the white matter of the brain towards the Schwann tissue at the cut end of the implanted nerve. These fibres, however, examined with a high-power objective, show none of the characteristic appearances of actively regenerating fibres. On the contrary, at the margins of the infiltrated zone, the persisting fibres of the white matter show many signs of degeneration, such as fragmentation, uneven thickenings and the formation of conspicuous globular retraction bulbs. Compared with the regenerating fibres of the implanted nerves, also, they are but lightly impregnated, and they show no tendency to extend out along adjacent strands of Schwann cells. At one point in their farthest extension the outgrowing facial nerve fibres actually intermingle in the granulation tissue with persistent fibres of the white matter (Pl. 1, fig. 3), and it may be difficult by superficial inspection of one section alone to determine the source and identity of some of them. However, a detailed study of the serial sections indicates that all those fibres which show characteristic signs of active regeneration and which extend along strands of Schwann cells are derived from the fibres of the facial nerve.

The proximal part of the outgrowth of axons and Schwann cells from the stump of the implanted nerve is in direct contact on one side with the white matter of the alveus, the lateral margin of which it penetrates. Here there is no inflammatory reaction in the tissue of the brain, and the alvear fibres are immediately contiguous with the proliferating Schwann elements at the end of the facial nerve with no intervening tissue (Pl. 1, fig. 4). Moreover, some strands of Schwann cells actually penetrate in among the fibres of the white matter. However, the latter show no tendency to extend along the Schwann cells with which they are in direct contact. The plexus of alvear fibres as a whole presents an abrupt margin where it meets the adjacent proliferating Schwann tissue, and even where the fibres almost touch outgrowing fibres from the implanted nerve they still show no tendency to make use of the strands of Schwann cells available to them for regenerative extension.

As the main part of the implanted nerve passes through the superficial layer of parietal cortex, the cut ends of some of the smaller fasciculi are seen to be imbedded in the cortex and also in the subcortical white matter. From these stumps regenerating fibres have grown out, but they do not penetrate into the normal tissue of the brain. They wander down closely hugging the surface of the perineural sheaths of adjacent fasciculi and are separated from the fibre plexuses of the cortex and white matter by a thin secondary capsule of fibrous elements derived from the brain tissues. Some of the outgrowing fibres have found their way into the substance of this capsule. On the other hand, the fibres of the adjacent cortex and white matter of the brain show no tendency to extend out towards the implanted nerve, nor are they found in the fibrous layer separating them from the latter.

R 233. Proximal stump of facial nerve inserted into brain after crushing, and animal killed 4 weeks later. Pyridine-silver sections show that the nerve extends through the parietal region of the cortex and the subcortical white matter, and through the dorsal part of the underlying hippocampus. Its free end lies on the inner surface of the brain imbedded in the leptomeninges between the hippocampus laterally and the optic tract medially (Pl. 2, fig. 7). Vigorous regeneration of the facial nerve fibres has occurred, the individual fasciculi being filled with large numbers of actively growing axons which are deeply impregnated. The tissues of the brain show remarkably little reaction.

At the free cut end of the nerve, many regenerating axons have emerged from the stump. Some of these become lost in the pial tissue covering the stump, a few running for a short distance alongside small pial veins. Most of them curl back up the surface of the nerve stump, pushing their way between the latter and the adjacent surfaces of the brain.

On the medial side they extend up on the surface of the hippocampus and on the lateral side on the surface of the optic tract (Pl. 1, fig. 6; Pl. 2, fig. 9). The superficial fibres of the latter, it should be noted, have been slightly traumatized by the lesion. These outgrowing fibres are accompanied by elongated cellular elements which appear to be proliferated Schwann cells, but it is not possible to identify them with certainty. Although the regenerating axons run alongside the alvear fibres of the hippocampus and the fibres of the optic tract, in neither case do these fibres of the brain show any tendency to deviate from this normal position and to extend out towards the regenerating fasciculi. In their passage through the subcortical white matter, some superficial fasciculi of the implanted nerve are in almost immediate contact with the former, and at one point the regenerating facial nerve fibres are separated from the fibre plexuses of the white matter by a distance of not more than 15μ (Pl. 2, fig. 8). Yet the fibres of the white matter show no evidence of new growth.

R 229. Proximal stump of facial nerve inserted into brain after crushing, and animal killed 3 weeks later. Pyridine-silver sections show that the nerve penetrates through the parietal area of the cortex and the subcortical white matter to reach the ventricular cavity. The cut end of the nerve lies free within the lateral ventricle in close relation to the ventricular surface of caudate nucleus, the fimbria and choroid plexus. The parotid duct was found to have been inadvertently implanted with the facial nerve. In the cortex and subcortical white matter immediately adjacent to the nerve there is considerable inflammatory reaction, accompanied by local necrosis and infiltration with macrophages. Regeneration of the facial nerve fibres has occurred, though not so abundantly as in the two previous experiments. Many of the regenerated fibres have reached the cut end of the implanted nerve, and most of them here turn aside and wander round within the confines of the perineural sheaths. At one point, however, some have grown out into the layer of granulation tissue which covers the nerve stump where it lies within the lateral ventricle. Of these, a few extend their course to the surface of the fimbria and even penetrate in among the most superficial fibres of this tract. Of particular note, however, are two or three stout fibres which, leaving the cut end of the implanted nerve, traverse a layer of granulation tissue to reach the choroid plexus. In relation to this structure they extend downwards and break up into a leash of very fine beaded fibres which pursue a tortuous course over the surface of the choroid plexus, being imbedded in a fine film of exudate which covers the choroid epithelium (Pl. 2, fig. 11). In the choroid plexus a relatively large, newly formed artery extends down towards the site of a localized lesion in the internal capsule which had evidently been produced at the time of operation. Alongside this artery, the regenerating nerve fibres have also extended their growth, and they finally beome lost in granulation tissue. So far as can be seen in the sections, the fibres which emerge from the implanted nerve are not accompanied by Schwann cells after leaving the granulation tissue immediately covering the cut end of the nerve. It may be noted that the epithelium of the implanted parotid duct has undergone considerable proliferation at one point, and has penetrated into the adjacent granulation tissue in a system of reticulating cords and scattered islands. At some places these epithelial outgrowths come into close contact with fibres of the white matter of the brain, and in two sections a few of these fibres are seen to be actually within an epithelial island.

R 228. Proximal stump of facial nerve inserted into brain and animal killed 1 week later. Pyridine-silver sections show the stump of the nerve passing through the parietal area of the cortex and the subcortical white matter, with its free end in contact with the fimbria of the underlying hippocampus. The fibres of the facial nerve have all undergone degeneration and no regenerating axons are present. The parotid duct had been inserted into the brain alongside the facial nerve and appears considerably dilated in the sections. The epithelial lining of the duct has undergone very active proliferation, penetrating in reticulating columns of cells into the adjacent cortex. This has led to degenerative changes in many of the fibres of the cortical plexuses which have been involved. At many points the invading epithelial strands, as well as the cellular reaction in the brain tissues, have displaced fibres of the cortical plexuses and have compressed them into compact fasciculi which might give the impression of an orientated growth of regenerating fibres. At other points in individual sections the network of invading epithelium seems to have cut off islands and recesses of cortical tissues in which many fibres of the cortical plexuses remain. Lastly, nerve fibres of the cortex are here and there actually found within the substance of epithelial clumps, lying among the constituent cells (Pl. 2, fig. 10). There is no evidence of the new growth of fibres of the brain in relation to the implanted portion of the facial nerve.

R 231. Proximal stump of facial nerve inserted into brain after crushing and animal killed 2 weeks later. The sections, treated by Bodian's protargol technique, show a condition very similar to that of the previous experiment. The nerve passes through the parietal cortex and penetrates into the underlying hippocampal formation. In one fasciculus only have any fibres of the facial nerve undergone regeneration, and here they are few and scattered. None of them can be traced as far as the cut end of the implanted nerve. There is no evidence of regenerative activity in intrinsic nerve fibres of the brain.

R 249. Distal stump of occipital nerve inserted into the brain and animal killed 3 weeks later. Pyridine-silver sections show that the nerve has traversed the occipital lobe where this overlaps the mid-brain and penetrates into the substance of the superior colliculus. There is very little reaction in the tissues of the brain except at the cut end of the implanted nerve where proliferated Schwann tissue has become extruded for a short distance beyond the limits of the epineural sheath into the damaged part of the colliculus. In this region there is some infiltration of immediately adjacent brain tissue with macrophages and an increased local vascularity. Damaged axons in the fibre plexus surrounding the stump of the implanted nerve are thickened, heavily impregnated and irregularly varicose (Pl. 2, fig. 12), and many of them end in large globular retraction bulbs (Pl. 2, fig. 13; Pl. 3, fig. 18). They show no evidence of active regeneration and no tendency to penetrate the implanted nerve or the proliferating Schwann tissue at its cut end. The implanted nerve is filled with healthy looking Büngner bands among which are scattered macrophages. Some fine actively growing fibres enter the implanted nerve

at its free extremity and can be traced along the elongated Schwann cells for a distance of just over 2 mm. (Pl. 2, fig. 14). Probably not more than four main axons have thus entered the nerve, but in their course they each divide into two or three separate fibres. Their precise origin cannot be determined. At the cut end of the implanted nerve they can be traced into the vascular tissue which is intermingled with proliferating masses of Schwann cells and here they become lost among intrinsic fibres of the superior colliculus which persist in the infiltrated zone. It may be noted that surrounding the lesion there is an appearance of fibres in the colliculus converging towards the stump of the implanted nerve (Pl. 2, fig. 12). This appearance is evidently the result of a secondary distortion produced by the lesion, and none of these fibres show any of the characteristic signs of active regeneration.

R 250. Distal stump of occipital nerve inserted into the brain and animal killed 4 weeks later. One week before death, the implanted nerve was cut close to the point where it enters the skull. Sections stained with the Bodian technique show that the nerve extends through the superficial cortex of the occipital lobe and the subcortical white matter, and penetrates into the cortex on the under surface of the occipital lobe (Pl. 3, fig. 15). The main cut end of the implanted nerve is actually imbedded in the deep cortical layers, and from it a compact outgrowth of Schwann tissue bulges out beyond the limits of the epineural sheath and presses against the upper surface of the superior colliculus. There is practically no inflammatory reaction in the surrounding brain substance, from which the nerve is separated only by a thin layer of delicate reticular tissue. The pial tissue overlying the cut end of the nerve is very vascular, and arterioles and capillaries can be traced from the under surface of the occipital lobe into the Schwann outgrowth. The implanted nerve appears in a healthy condition with a strong development of Büngner's bands. In a few sections newly growing axons are seen; they are extremely fine, usually beaded, and run a slightly tortuous course in close relation to elongated Schwann cells. In some cases they end in a conspicuous thickened bulb from which two or three fine sprouts extend out. A study of the serial sections show that probably not more than three separate main fibres have entered the implanted nerve, and they all lie in the same region near the surface. These fibres are found to penetrate the nerve from the proliferated Schwann tissue at the cut end. They can be traced along the implanted nerve for a distance of 1·2 mm. In one section a fine fibre can be seen curling round from the pial tissue on the under surface of the occipital lobe to enter the tip of the Schwann outgrowths, and it can be traced proximally to a plexus of apparently newly formed capillary vessels in the overlying pia. In another section a nerve fibre is seen entering the stump of the nerve alongside a small arteriole, dichotomizing as it does so in a manner characteristic of regenerating axons. The arteriole itself is derived from the vascular plexus in the pia, and it should be noted that in the tunica adventitia of some of the small arteries on the surface of the cortex vascular nerve fibres are distinctly impregnated. It thus appears almost certain that the few fine axons which have entered the implanted nerve are derived from vascular nerve fibres. There is no evidence that any of the intrinsic fibres of the white matter or cortex of the brain extend into or even towards the cut end of the implanted nerve. In the absence of inflammatory reaction, also, there is no distortion of the plexuses of the brain and there is also no tendency for orientation of their constituent fibres in the direction of the proliferated Schwann tissue.

R 254. Distal stump of occipital nerve inserted into the brain and animal killed 5 weeks later. Pyridine-silver sections show that the nerve passes through the occipital cortex and its cut end lies in the white matter of the subjacent subicular cortex. The tissues of the brain show a remarkable absence of inflammatory reaction and there is no apparent fibrosis around the nerve. The latter is filled with vigorous and healthy looking Schwann cells, neatly alined in characteristic band formation. From the cut end of the nerve, proliferated Schwann tissue fans out in a compact mass which presses against the adjacent cortex and reaches the pial tissue covering the under surface of the occipital lobe (Pl. 3, fig. 16). Under the high power, some of the Schwann cells appear to penetrate into the brain tissue to come into direct contact with fibres of the cortical plexuses. Others extend up along the superficial aspect of the epineural sheath between it and the surrounding brain tissue. Regenerating axons are scattered throughout the implanted nerve in somewhat surprising abundance. A study of the serial sections shows that they have grown downwards from the peripheral part of the nerve outside the skull, and they are therefore presumably recurrent fibres which may normally occur in the trunk of the occipital nerve. They can be followed down to the cut end of the nerve where they issue out into the proliferated mass of Schwann tissue. Here their further course is variable. Many become interwoven in a tangled skein within the 'Schwannoma' at the cut extremity of the nerve. Others, having emerged from the nerve trunk, turn back and run upwards on the surface of the epineurium for a considerable distance. One or two fine fibres penetrate into the pial tissues on the under surface of the occipital lobe, but their course here is very short. It may be noted that these regenerating fibres at many points come into the closest relation with intrinsic fibres of the adjacent cortex, but they never intermingle with them. The degree of impregnation of the material from this experiment is particularly favourable for following individual fibres section by section; yet in no instance could any fibre be found passing from the tissues of the brain into the implanted nerve. There is also no evidence of any orientation of fibres in the brain towards the Schwann proliferation. Further, it is to be remarked that the traumatic reactions of fibres in the brain which are so conspicuous in Exp. R 249 (such as thickening, heavy impregnation, varicosities and retraction bulbs) are almost completely absent in the present case. Presumably this is due to the longer interval which has allowed time for the resorption of fibres damaged at the time of the operation, and also to the almost complete absence of inflammatory reaction and cellular infiltration in the brain.

R 255. Distal stump of occipital nerve inserted into the brain and animal killed 6 weeks later. Pyridine-silver sections show that the nerve has traversed the occipital lobe of the cerebral hemisphere, and its free end lies in the pial tissue at the surface of the deep layer of cortex, in contact with the upper surface of the superior colliculus. The brain tissue in which the nerve lies shows almost no

reaction. The nerve is filled with closely packed Schwann cells in band formation, and but few macrophages remain (Pl. 3, figs. 17, 19). Extending along the nerve are many scattered fibres in process of regeneration, but they are considerably fewer than those in the previous experiment. By tracing these fibres through the serial sections they are all found to have travelled down in the nerve from outside the skull—that is, they are recurrent fibres. At the cut end of the nerve there is a small opening in the cap of fibrous tissue covering it, and though this is a slender outgrowth of Schwann cells, accompanied by a few regenerating fibres, has pushed its way towards the colliculus. The colliculus itself shows a circumscribed puncture lesion extending down for a few millimetres into its substance from the position of the free end of the nerve, which was evidently produced at the operation. Along the track of the lesion some fine strands of Schwann cells have penetrated into the colliculus and with them, also, a few of the regenerating fibres. The latter become extremely fine as they are traced down, until they pass beyond the limits of visibility. In immediate contact with this extension of Schwann tissue (with no intervening fibrous layer) are the fibre plexuses in the superficial layers of the superior colliculus. The component fibres appear inert (Pl. 3, fig. 20). Very occasionally a small retraction bulb is seen, and at the margin of the lesion a few fibres have become displaced by the proliferated Schwann cells, but there is no histological evidence of regenerative activity.

DISCUSSION

The results of the experiments recorded above may be briefly discussed in relation to two main points —the behaviour of regenerating axons which grow down the inserted nerve towards the brain tissue, and the evidence for regenerative activity on the part of the intrinsic fibres of the brain.

The great majority of the regenerating fibres which grow down peripheral nerve stumps inserted into the brain do not extend beyond the confines of the implanted nerve and the compact mass of Schwann cells ('Schwannoma') which develops at its free extremity. Many are met by the obstruction of fibrous tissue at the cut end of the nerve and, running spirally around the inner surface of the perineural sheaths, form complex entanglements which often extend back up the nerve trunk for a little distance. Those fibres which enter the Schwann outgrowth at the cut end of the nerve in most cases become coiled round and interwoven with each other in a loose skein. Some regenerating fibres, however, may emerge from the cut end of the nerve and extend their growth into the surrounding tissues. Usually they turn aside from their original course, returning along the outer surface of the perineural sheaths of the implanted nerve and in immediate relation to the surrounding brain tissues. In this position regenerating fibres of the facial nerve have been observed running over the surface of the hippocampus and the optic tract, or alongside cut margins of cortical tissues or white matter of the brain. They may be separated from the latter by a thin secondary capsule, and in some cases they actually penetrate among the fibrous elements of this capsule. In one experiment there was a very conspicuous outgrowth of facial nerve fibres into a zone of granulation tissue in the adjacent part of the brain, and a significant feature in this case was the intermingling of the regenerating fibres with surviving fibres of the white matter. Another interesting observation is provided by the experiment in which regenerating facial nerve fibres had extended on to the choroid plexus in the lateral ventricle and had grown down in a convoluted course for some distance over the surface of the choroid epithelium. It may be noted that it was not possible to determine the presence of Schwann cells in relation to some of these finer extensions of the regenerating fibres. In no instance was any evidence found of regenerating facial nerve fibres penetrating into normal brain tissue in which no cellular infiltration was present.

Evidence which might indicate regeneration of intrinsic nerve fibres of the brain may be discussed under the following headings:

Growth of fibres into the implanted nerves. In two experiments only, in both of which the occipital nerve had been inserted into the brain, were newly regenerated axons found to enter the nerve from the brain tissues. In both these cases such regenerating fibres were very fine and extremely few—four main axons in one and three in another. In one case they were found to enter the Schwann outgrowth in company with small blood vessels from the pial tissue on the surface of the brain, and it was apparent that they were extensions of the vascular nerve fibres found in the tunica adventitia of cerebral arteries. In the other case they were traced proximally to newly vascularized tissue at the site of a lesion in the superior colliculus, and, though their precise origin could not be demonstrated, the sections provided no evidence that they had been derived from the intrinsic fibres of the brain. On the contrary, it seems probable that in this case, also, they had regenerated from vascular fibres. It is concluded, therefore, that the growth of new axons from the brain into the implanted nerves which was observed in these experiments almost certainly involves only extrinsic fibres accompanying blood vessels. This accords with observations previously made on predegenerated nerve grafts (Clark, 1942).

Orientation of intrinsic fibres of the brain towards the implanted nerves. In the previous communication, attention was drawn to Tello's observation (1911) that fibres of the white matter of the host brain converged towards an implanted fragment of predegenerated nerve as though attracted towards it, and to his inference that this appearance indicated a directed growth under the influence of the grafted tissue. The observations of Tello were

confirmed, but it was suggested that the orientation of the fibres is partly the result of a traction effect imposed on the host tissue by the graft, and partly due to the dislocation of pre-existing fibres by the infiltration of the brain with granulation tissue. In the present series of experiments, a similar orientation of fibres has been found only in those cases in which local inflammatory reaction of the brain is marked, and a study of the sections makes it clear that the fibres are not in a state of active regeneration but are pre-existing fibres distorted by the initial lesion or by cellular infiltration. In some cases remains of the fibre plexuses of the brain had been compressed into compact and apparently orientated fasciculi between small masses of granulation tissue, or, in those experiments in which the parotid duct had been inserted with the nerve, by proliferating masses of epithelial cells. In other cases the survival of fibres of the brain plexuses alongside blood vessels in the infiltrated tissue also gives rise to an appearance of orientated growth which is certainly fallacious. Lastly, it must be emphasized that, in those experiments in which there is little or no damage to or reaction in the surrounding brain tissue, no orientation of the intrinsic fibres of the brain towards the implanted nerve or the Schwann proliferation is found. Since in these cases the Schwann elements of the implanted nerve are seen histologically to be in a vigorous and active condition, it may be assumed that they are not capable by themselves of exerting an attractive influence on the intrinsic fibres of the brain.

Morphological characters of fibres of the brain immediately adjacent to the implanted nerves. As in the previous series of experiments, fibres of the brain in immediate relation to the implanted nerve have in some cases been found to show striking traumatic changes in their detailed configuration, such as thickening, irregular varicosities, end-swellings, deviations from their normal direction, granulation and so forth. There is little doubt that some of these changes have occasionally been regarded by neurohistologists as evidence of regeneration, or at least of attempted or obstructed regeneration. However, there is no clear reason to suppose this is so. Retraction bulbs are well recognized to be degenerative phenomena (Cajal, 1928), and they are usually to be distinguished from thickenings resulting from obstruction at the advancing ends of regenerating fibres by their coarseness and their globular outline. The varicosities of degenerating axons often resemble the beading of growing axons, but they are commonly less regular and less sharply outlined. Lastly, the displacement of nerve fibres by the immediate pressure of cellular proliferation and infiltration is to be distinguished from the aberrant wanderings of newly regenerated fibres. It should be noted that, in the experiments in which the implanted nerve had been left in position for 5 and 6 weeks, and in which the surrounding brain tissue had suffered minimal damage and showed an almost complete absence of inflammatory reaction, the fibres of the brain adjacent to the nerve were almost completely devoid of irregularities which might be taken as evidence of regenerative activity.

Relation of fibres of the brain to epithelial proliferation. It has been noted that in those experiments in which the parotid duct had been inserted into the brain alongside the facial nerve, the epithelial lining of the duct shows considerable proliferative activity, sending out reticulating columns of cells which invade the adjacent cortical and white matter of the brain. Further, nerve fibres derived from those of the brain are in a few sections found to be situated within epithelial clumps, among their constituent cells. This appearance might be taken as evidence of regenerative growth on the part of the brain fibres, leading to their active penetration into the epithelial masses. However, a careful study of the material makes it reasonably certain that they are really pre-existing fibres which have become secondarily caught up and included within the advancing columns of cells. Where the latter are found to contain nerve fibres, the cellular elements are somewhat loosely arranged, and the histological appearance makes it easy to understand how the rapidly proliferating epithelium is able to surround and occasionally enclose a few fibres. The latter, it should be noted, show none of the characteristic morphological features of regenerating fibres. On the contrary, they are clearly in process of degeneration for many of them are fragmenting and are accompanied by a granular argentophil deposit, while others terminate in swollen retraction bulbs. In connexion with these observations, reference may be made to experiments by Shirai (1935) in which pieces of skin were transplanted into the brains of rabbits. This author believed that active regeneration of nerve fibres in the brain occurred, leading to penetration and innervation of the transplanted skin, sometimes with the formation of a differentiated end-apparatus. However, from his descriptions and photomicrographs, it is hardly possible to avoid the conclusion that the fibres observed by him were similar to those seen in relation to the proliferating epithelium in the present series of experiments. Secondary inclusion was evidently mistaken for active penetration, and retraction bulbs were mistaken for differentiated end-formations. The fact that Shirai found that all the 'regenerating' fibres had disappeared after 100 days further supports this interpretation.

The results of all the experiments recorded in this report reinforce the general impression gained from the previous series of experiments that intrinsic fibres of the rabbit's brain probably do not possess the capacity for true regeneration. Clearly it is a matter of great difficulty completely to prove that this is the case, since it remains possible that under experimental conditions other than those which

have hitherto been employed regenerative growth might possibly occur. There is reason to believe, however, that in the present series of experiments optimal conditions for inducing regeneration of nerve fibres in the brain have been provided; the implanted nerves were in effect autografts in which a vascular supply was maintained, the Schwann elements showed normal and healthy activity and came into the closest relation with the nerve fibres of the brain, in most cases the surrounding brain tissue showed remarkably little inflammatory reaction, and the inserted nerves clearly provided perfectly good facilities for a vigorous regeneration of extrinsic fibres growing downwards towards the brain. In view of these considerations it may be accepted that the negative evidence presented by the experimental material is highly significant.

In general, in the assumption that the fundamental identity in structure of axons in the central and peripheral nervous system implies a similar capacity for regeneration under suitable conditions, two explanations are commonly put forward to account for the normal absence of regeneration in the brain and spinal cord of higher vertebrates during post-natal life. One is the absence in the central nervous system of Schwann cells which are believed to facilitate regeneration either by some chemotropic action or by the provision of suitable surfaces along which the growth of axons can proceed. The other is the density and texture of the tissues of the central nervous system, which impose an insuperable obstacle to fibres which may be attempting regeneration. In regard to the presumed influence of the Schwann cells, it may be emphasized again that at many places in the experimental material these elements were seen to be in direct contact with fibre plexuses of the brain, yet these fibres showed no tendency to extend towards or along them. The same lack of response was shown in places where Schwann bands, accompanied by actively growing peripheral nerve fibres, were found to pass in the closest relation to the cortex and white matter of the brain with no intervening barrier of fibrous tissue that could be defined histologically. The probability that the tissues of the central nervous system are not suitable for the growth through them of regenerating nerve fibres in post-natal life is indicated by the fact that in no case were the regenerating fibres growing down the implanted nerves found to penetrate into normal brain tissue. On the other hand, facial nerve fibres were found to extend out into zones of cellular infiltration still containing persistent fibres of the white matter (freely intermingling with the latter), or on the surface of the choroid plexus in the lateral ventricle, or on the surface of the optic tract and hippocampus, and also among the fibrous elements of secondary capsules which had been formed in the brain tissues immediately surrounding the implanted nerve. Similar paths were of course available for the outgrowth of injured fibres of the brain if the latter are indeed capable of regeneration. It may be concluded, therefore, that the normal absence of regeneration in the central nervous system is fundamentally due neither to the absence of Schwann cells nor to the physical obstruction provided by the texture of the nervous matrix. In regard to the second factor, attention may be drawn to a study made by Clark (1914) on nerve regeneration in fowls following experimental non-traumatic degeneration. The degeneration was produced by prolonged feeding on polished rice, and regeneration by a return to an adequate diet. While new outgrowths of axons took place in the peripheral nerves, no regeneration occurred in the spinal cord. Yet the glial sheaths of individual nerve fibres in the cord, containing globules of degenerated myelin, apparently persisted for more than a year, and it seems that these sheaths would provide adequate pathways for new outgrowths of axons if the intrinsic fibres of the spinal cord were capable of regeneration.

SUMMARY

In a series of rabbits the proximal stump of the cut facial nerve or the distal stump of a cut occipital nerve was inserted into the brain and left in position for 1 to 6 weeks. As compared with the implantation of homografts of predegenerated sciatic nerves recorded in previous experiments, this method combines the advantages of an autograft with the retention of at least a part of the vascular supply. Reaction in the tissues of the brain was reduced to a minimum, and it is claimed that the optimal conditions were provided for the induction of the growth of intrinsic fibres of the brain if these are capable of regeneration like the peripheral nerve fibres. In some experiments proliferated Schwann tissue had extended out from the cut end of the nerve into the adjacent tissues of the brain. These Schwann outgrowths may be accompanied by regenerating fibres which had grown down in the implanted nerve from outside the skull. Such regenerating peripheral nerve fibres may intermingle freely with intrinsic fibres of the brain, in areas of cellular infiltration. They have also been observed extending up the outer surface of the perineural sheath of the nerve, over the surface of the optic tract and the hippocampus, and also over the surface of the choroid plexus in the lateral ventricle. In two experiments three or four regenerating axons of very fine diameter were found to have entered the cut end of the implanted nerve from the brain tissues, but these were almost certainly derived from vascular nerves accompanying cerebral blood vessels, and not from intrinsic fibres of the brain. In none of these experiments was any evidence found of regenerative activity on the part of the nerve fibres of the brain, in spite of the fact that they may come into the closest relation with

actively proliferating strands of Schwann cells and with regenerating peripheral nerve fibres. As in previous experiments, some sections gave the appearance of a convergence towards the end of the implanted nerve of fibres in adjacent regions of the brain, This appearance, however, was not found in the absence of traumatic reaction in the tissues of the brain, and it is believed to be the result of dislocation of pre-existing fibres and not indicative of active growth. In some experiments in which the parotid duct had been inserted into the brain along with the facial nerve, the proliferating epithelium of the duct had invaded the adjacent tissues of the brain. No evidence was found of the regeneration of fibres of the brain in relation to the epithelial masses. The negative evidence of the histological material is believed to justify the conclusion that the intrinsic fibres of the rabbit's brain show no capacity for regeneration under the conditions of the experiments.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. The stump of the facial nerve, inserted into the brain 3 weeks previously (R 230, 28·6). The cut end of the perineural sheath is marked *a*, and from it numerous fasciculi of regenerating axons are seen streaming out into an area of granulation tissue which infiltrates the adjacent region of the brain. Above (*b*) are seen the fibres of the white matter of the brain; they show no regenerative activity in relation to the outgrowth of facial nerve fibres. × 130.

Fig. 2. A higher power view of the regenerating fibres seen in the previous figure (R 230, 26·10). The fibres are seen to be extending along strands of Schwann cells which have grown out from the cut end of the nerve into the granulation tissue. × 380.

Fig. 3. Some of the terminal sprouts (*a*) of the regenerating facial nerve fibres in the same experiment (R 230, 26·9). Above and to the right are seen numerous persisting fibres of the white matter of the brain; these show fusiform varicosities but no signs of regenerative activity. The intimate relation between the brain fibres and the actively growing fibres of the facial nerve should be noted. × 380.

Fig. 4. A fasciculus of regenerating fibres (*a*) in R 230 (27·3), extending along a strand of Schwann cells in close relation to fibres of the white matter of the brain (*b*). The latter show no tendency to grow out in relation to the regenerating fibres. × 430.

Fig. 5. Longitudinal section of one of the fasciculi of the facial nerve in R 230 (27·6), showing the usual fate of the regenerating fibres. At the end of the perineural sheath they form a tangled skein from which many fibres extend backwards again, winding in a spiral manner on the inner aspect of the sheath. × 200.

Fig. 6. This illustrates a skein of fine, actively regenerating fibres of the facial nerve (*a*) growing along the surface of the hippocampus in the region indicated by the right arrow in Pl. 2, fig. 1 (R 233, 34·10). To the left are seen the alvear fibres at the surface of the hippocampus. × 360.

Plate 2

Fig. 7. The stump of the facial nerve in R 233 (40·4), projecting down on the medial aspect of the cerebral hemisphere between the optic tract (*a*) and the hippocampus (*b*). Fasciculi of regenerating fibres can be seen in the stump. The arrows mark the regions where fibres after growing out from the cut end of the nerve have extended up on the surface of the hippocampus (see Pl. 1, fig. 6) and on the surface of the optic tract (see Pl. 2, fig. 9). × 32.

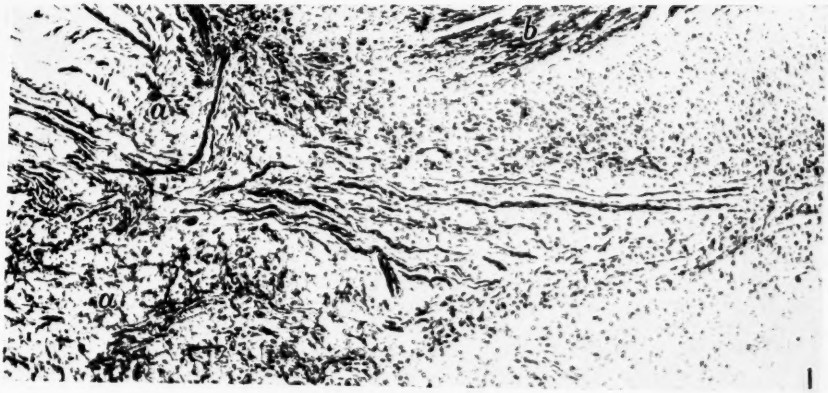
Fig. 8. R 233 (39·10). A small fasciculus of regenerating facial nerve fibres (right) passing in close proximity to the fibres of the white matter of the brain (left). The latter show no tendency to extend out towards the regenerating fibres. × 230.

Fig. 9. High power view from R 233 (41·10) showing regenerating facial nerve fibres (centre and right) growing along the surface of the optic tract (*a*), in the region indicated by the left arrow in Pl. 2, fig. 7. × 240.

Fig. 10. A proliferating mass of parotid duct epithelium in R 228 (20·8). In the centre a nerve fibre is seen extending up into the mass, and to the right is a small fasciculus of fibres in close relation to the epithelial cells. These fibres show no histological characteristics of active regeneration; they are evidently pre-existing fibres which have become secondarily included in the proliferating columns of cells. × 360.

Fig. 11. A skein of fine regenerating fibres of the facial nerve in R 229 (28·8), which have grown down on the surface of the choroid plexus in the lateral ventricle. They are imbedded in a thin film of exudate which covers the choroid epithelium. × 230.

Fig. 12. Section from R 249 (25·9), showing the relation of the proliferated Schwann tissue at the extremity of the implanted occipital nerve to the fibres of the superior colliculus. Below and to the right are seen the bands of Schwann cells. At the margin of the Schwann tissue the fibres of the superior colliculus stop abruptly and show no tendency to invade the tissue. Many of them show traumatic reactions such as irregular thickenings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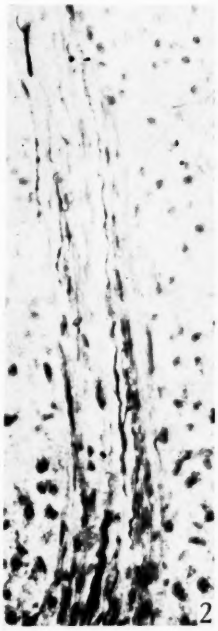
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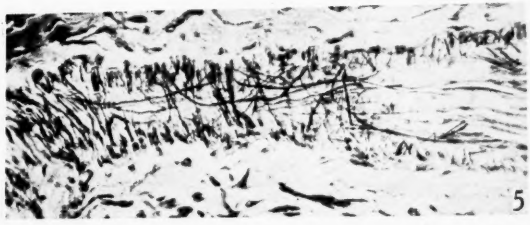
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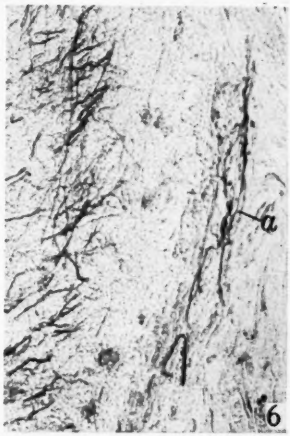
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and retraction bulbs. To the left the fibres of the superior colliculus seem to stream towards the outgrowth of Schwann cells. This is a secondary distortion produced by the lesion, and none of the fibres show histological characters of active regeneration. ×290.

Fig. 13. High-power view showing traumatic reaction in fibres of the superior colliculus in R 249 (22·2). A fibre is seen ending in a conspicuous retraction bulb at the margin of the Schwann outgrowth (above). To the right many traumatized fibres are shown with irregular varicosities. ×410.

Fig. 14. Two fine regenerating fibres in the implanted occipital nerve in R 249 (21·10). They have grown into the nerve from the brain tissues and are almost certainly derived from vascular nerves. The fibres can be seen extending along in close relation to Schwann cells. They show beading, and the lower fibre dichotomizes at a local thickening. ×410.

PLATE 3

Fig. 15. Low-power view of the stump of the occipital nerve in R 250 (14·5), imbedded in the cortex. The Schwann outgrowth at its free end comes into contact with the upper surface of the superior colliculus (*a*). ×24.

Fig. 16. The free end of the occipital nerve in R 254 (13·5). Above is seen the stump of the nerve, and from it a mass of proliferating Schwann tissue has grown out into contact with the adjacent brain tissue. In the Schwann outgrowth can be seen some regenerating nerve fibres which have grown down in the nerve from outside the skull. Below and to the left some fibres of the cortical plexuses are visible. They show no tendency to invade the Schwann tissue. ×58.

Fig. 17. Section of the implanted occipital nerve in R 255 (23·8). Note the closely packed Schwann cells, and some regenerating fibres which have grown down into the nerve from outside the skull. ×240.

Fig. 18. Fibres showing traumatic reactions at the margin of the Schwann outgrowth in the superior colliculus in R 249 (22·9). The fibre to the left shows a globular retraction bulb, and that to the right is markedly thickened. The nuclei of Schwann cells can be faintly discerned above. ×410.

Fig. 19. Schwann bands in the occipital nerve in R 255 (22·8), 6 weeks after its implantation. Macrophages have now mostly disappeared. A fasciculus of regenerating fibres is seen; these have grown down in the nerve from outside the skull. ×240.

Fig. 20. Section through a puncture lesion in the superior colliculus in R 255 (22·14). The site of the lesion is occupied by granulation tissue into which a few Schwann cells from the cut end of the implanted occipital nerve have penetrated. Accompanying these cells are some extremely fine regenerating fibres which have grown down in the nerve from outside the skull. The position of one of these fibres is indicated by the arrow. On either side of the lesion are seen fibres of the superior colliculus. Some have been displaced by the lesion, but none of them show any signs of regeneration. Note that now (6 weeks after implantation) traumatic reactions of individual fibres have disappeared. ×160.